Adjuvants for Agrichemicals

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CRC Press
Boca Raton Ann Arbor London Tokyo

Library of Congress Cataloging-in-Publication Data

Adjuvants for agrichemicals editor, Chester L. Foy.
p. cm.
Includes bibliographical references and index.
ISBN 0-8493-6317-9
1. Agricultural chemicals-Adjuvants. I. Foy, Chester L.
S587.7.A36 1992
632 .95 — dc20

91-35158
CIP

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

International Standard Book Number 0-8493-6317-9

Library of Congress Card Number 91-35158

Printed in the United States of America 1 2 3 4 5 6 7 8 9 0

Printed on acid-free paper

FOREWORD

In 1977, Dr. Cortez F. Enlowe, Jr. described agriculture in North America as the "eighth wonder of the world." Dr. Enlowe pointed out that abundant food supplies were the single most important factor in the overall advancement of the United States and Canada, giving their people the freedom to provide the central support for Western Civilization. He concluded, "the most magnificent achievement of mankind is the creation in North America of the greatest land of nutritional abundance in the world. Not even the building of the Egyptian Pyramids, the writing of the Greek Classics, the painting of the masterpieces, the composition of the symphonies, the invention of steam power, or the splitting of the atom could fill the shadow of this towering triumph of the human spirit. It alone is the greatest thing man has ever done."

This accomplishment discussed by Dr. Cortez would not have been possible without the effective use of the many highly effective agrichemicals used to control pests in modern agriculture. The importance of adjuvants to agriculture parallels that of pesticides themselves. Most herbicides and other pesticides require surfactants or other adjuvants to maximize their efficacy or utility, either in the formulated product or as an adjuvant added to the spray tank.

Agriculture is in a state of transition at a time when the world has at least 90 million new mouths to feed each year. Three of the major forces that will cause significant changes in worldwide food production are (1) the ongoing loss of land devoted to food production; (2) the increased emphasis on alternative, sustainable, or low input agriculture; and (3) the present and continuing threat of loss of registration of agrichemicals important to agriculture. Several major conservation programs were established by the U.S. Congress in the 1985 Food Security Act that include The Conservation Reserve Program, Sodbuster, Swampbuster, Conservation Compliance, and the Acreage Reduction Program. These and similar programs in other countries, especially in Europe, will result in the loss of several hundred million acres of crop land if goals are met.

Many authorities agree that increased adoption of alternative agriculture may reduce crop yields on a per unit-of-land basis. The number of registered pesticides is being reduced by state and federal regulatory actions and by private sector decisions to withdraw individual products. Public concern and regulatory complexity are likely to increase in the future causing this trend to continue. All of these forces put increased pressure on agriculture to produce an abundant food supply with fewer resources. Increased efficiency through the use of surfactants and other adjuvants will play an ever increasingly important role in maintaining our food supply.

Advances in our understanding of improved formulations, application technology, and efficient use of adjuvants to improve the efficacy of agrichemicals remains one of the major ways in which food and fiber production can be sustained with fewer agrichemicals. New complex multi-phase systems are being developed for formulations that include micro-emulsions/suspensions, multiple formulations, and other new types of emulsions. These new systems will require significant advances in the use of surfactants and other adjuvants. The use of adjuvants to improve biological activity remains poorly understood in terms of the modes of action involved. Structural activity relationships for adjuvants continue to be elusive and there is ample room for advancements in this area. One of our most urgent needs is a bioassay that can provide a predictive capability of the comparative activity of surfactants and other adjuvants with specific pesticides under field conditions. The symposium leading

to this publication and future symposia on adjuvants and agrichemicals will play an ever-increasing role in providing more effective evaluation techniques and in increasing the efficiency of adjuvants. This publication and future volumes will provide a unique resource not only for scientists in the pesticide disciplines but also for other scientists as well. The authors and editors are commended for this major effort for making this expertise on adjuvants available for use by others.

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PREFACE

An estimated 50 percent or more of the world's production of food is lost to weeds, insects, diseases, and other factors. Much progress in crop protection has been made possible by the use of pesticides and other agrichemicals. Adjuvants are important to the production, marketing, application, and effective use of these chemicals. There are now over 200 pesticides registered by the U.S. Environmental Protection Agency that have specific recommendations for the use of adjuvants.

Although adjuvants have been commercially available for about 30 years, they may well be the most misunderstood crop protection products on the market today. Research is required to obtain basic information on the mode(s) of action of adjuvants and to develop practical applications of these research findings for agriculture.

The Second International Symposium on Adjuvants for Agrichemicals, held in Blacksburg, Virginia on July 31 to August 3, 1989, was designed to build upon the success of the first symposium held in 1986 in Brandon, Manitoba, Canada. Approximately 275 individuals participated in the second symposium, including 234 university faculty members and graduate students, corporate and association representatives, government regulatory officials, and private consultants. Twenty-four Asian, European, and North American countries were represented and over seventy-five scientific papers were presented.

This book, based on the second symposium, presents the topics addressed which include a bibliographic survey of research and development of agri-adjuvants; regulation and importance of adjuvants; rationale for adjuvant use; concerns within the pesticide industry relating to adjuvants; a review of the methods employed in laboratory evaluation of adjuvants; results of current research on various adjuvants (including organosilicone surfactants, oils, and emulsifiers) with herbicides, fungicides, insecticides, or growth regulators; and field, greenhouse, and laboratory methods for evaluating adjuvants. It is hoped that these topics will be of interest to many and that this book will promote a better understanding of the effects of adjuvants on pesticide penetration, translocation, photodegration and stability, spray deposition and dissipation, and the fate of herbicides in the environment. Also, the Organizing Committees of the first and second symposia revised and updated "Formulations and Applications of Adjuvants for Agrichemicals: A Selected Bibliography of World Literature in English" for use by agricultural researchers and others.

Chester L. Foy

THE EDITOR

Dr. Chester L. Foy is now Professor of Plant Physiology and Weed Science at Virginia Polytechnic Institute and State University, Blacksburg. He received the B.S. degree from the University of Tennessee, M.S. degree from the University of Missouri, and Ph.D. from the University of California-Davis. He joined Virginia Polytechnic Institute as Associate Professor in 1966, was promoted to Professor 1968, and served (1974 to 1980) as Head of the Department now known as Plant Pathology, Physiology, and Weed Science. Formerly he was at the University of California-Davis as Associate Botanist and Associate Professor of Agricultural Botany (1964 to 1966), Assistant Botanist (1958 to 1964), and Graduate Assistant (1953 to 1956). Earlier he was Assistant Instructor (Field Crops) at the University of Missouri (1952 to 1953).

Dr. Foy is a charter member of the Weed Science Society of America (WSSA) and the International Weed Science Society (IWSS). He is currently (1991-93) serving as President of IWSS and has served as President of WSSA. His affiliations with other professional organizations past and present include American Society of Agronomy, American Association for the Advancement of Science, American Institute of Biological Sciences, American Society of Plant Physiologists, Council of Agricultural Science and Technology, International Congress of Plant Protection, International Congress of Pesticide Chemistry, Plant Growth Regulator Society of America, Southern Weed Science Society (SWSS), Torch International, and Virginia Academy of Sciences. Dr. Foy's recognitions include election to membership in several academic honorary societies, and several "Who's Who" listings. Other awards for professional achievement include National Academy of Sciences Resident Research Associate Award, Gamma Sigma Delta Faculty Research Award, WSSA Outstanding Paper Award, WSSA Fellow, SWSS's first "Weed Scientist of the Year" Award, and WSSA's "Outstanding Researcher" Award.

Dr. Foy served as Editor of *Reviews of Weed Science* and is currently serving as Editor of *Weed Technology*. He is a charter (and current) member of the editorial board of the international journal, *Pesticide Biochemistry and Physiology*, and has served many years as WSSA Associate Editor and Reviewer for *Weed Science*, as well as Reviewer for several other scientific journals.

Dr. Foy conducts and directs field, greenhouse, and laboratory studies in the following areas: crop production and protection; vegetation management in agronomic and fruit crops, and control of specific perennial weeds; routes and mechanisms of absorption, translocation, accumulation, and exudation of herbicides and growth regulators, surfactants, and other adjuvants; metabolism and fate of these substances; physiological, biochemical, and morphological changes induced by exogenous chemicals; modes of action and selectivity of herbicides and growth regulators; minimizing pesticide residues in the biosphere; allelopathy; and parasitic weeds. His publications include 21 book chapters; 5 book reviews; more than 90 peer-reviewed scientific journal papers; 5 technical research bulletins; over 340 contributions to conference proceedings, research reports, abstracts or scientific papers, and special technical research articles; and more than 200 semi-technical or extension publications.

ACKNOWLEDGMENTS

The Second International Symposium on Adjuvants for Agrichemicals was sponsored by the Department of Plant Pathology, Physiology, and Weed Science (PPWS), Virginia Polytechnic Institute and State University, Blacksburg, with supplemental support from representatives of the agricultural industry and other sources. The Editor (Symposium Chairman) gratefully acknowledges the following organizations for supplemental financial support: American Cyanamid Company, Aquatrols Corporation of America, BASF Canada, Inc., BASF Corporation (U.S.A.), Brandon Research Station (Manitoba, Canada), Ciba-Geigy Corporation, Dow Chemical U.S.A., E. I. duPont de Nemours and Company, Eli Lilly and Company, Exxon Company U.S.A., Fermenta ACS Corporation, Helena Chemical Company, Loveland Industries, Inc., Monsanto Canada, Inc., Rohm and Haas, and Sandoz Crop Protection. Thanks are extended to other members of the Organizing/Coordinating/Advisory Committee: Paul N. P. Chow and Cynthia A. Grant, Brandon Research Station, Manitoba, Canada; Peter J. Holloway, Long Ashton Research Station, University of Bristol, Bristol, England; Allen Underwood, Helena Chemical Company, Memphis, TN; Bob Reeves, Loveland Industries, Inc., Greeley, CO; and Stephen Scheneman, Donaldson Brown Center, Virginia Tech, Blacksburg, VA. Appreciation is extended to members of the PPWS departmental support staff, Judy Fielder, Missy Hawley, Jeanne Keister, Judy Massey, Ann Rader, Lori Rogers, and Harold Witt, for typing and other assistance.

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ADJUVANTS AND AGROCHEMICALS*

Volume I
MODE OF ACTION AND PHYSIOLOGICAL BASIS

Volume II
RECENT DEVELOPMENT, APPLICATION, AND BIBLIOGRAPHY OF AGRO-ADJUVANTS

* Volumes from the First International Symposium on Adjuvants for Agrochemicals in Brandon, Manitoba, 1986. Published by CRC Press in 1989.

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A Bibliographic Survey; Physiological Action; Effects on Pesticide Penetration, Translocation, Photodegradation, and Stability

Chapter 1

RESEARCH AND DEVELOPMENT OF AGRO-ADJUVANTS: A BIBLIOGRAPHIC SURVEY

Paul N. P. Chow and Cynthia A. Grant

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ABSTRACT

This bibliographic survey reflects the research and development of agro-adjuvants in the past and today. In view of concerns on economic stress and chemical residues in the environment, future research and development of agro-adjuvants should emphasize renewable, biodegradable, and inexpensive raw materials such as crop oils and carbohydrates. New adjuvants should be more efficient, nontoxic, have less impact on the environment, and maximize the activity of pesticides. However, research efforts should not ignore an investigation of the metabolism and mode-of-action of adjuvants per se, their physiological effects on plants, and their role in pesticide formulation and application.

I. INTRODUCTION

Recognized pesticides have contributed greatly to the reduction of crop losses, resulting in increased crop yields and lower crop production costs. It is estimated that farmers in western Canada used 17.5 million kilograms of herbicides to control weed pests in 1985, and American farmers applied 182 million kilograms of herbicides and spent $3.6 billion for chemical weed control in 1976. All herbicides require adjuvants to maximize the efficiency of their herbicidal activity, thereby reducing the rate of active ingredients entering the environment. Close cooperation among industry, universities, and government research organizations have, needless to say, contributed greatly to the successful development of pesticides, adjuvants, and other agrochemicals.

A vast amount of literature on agro-adjuvants and pesticides has been published every year in international scientific journals, monographs, and proceedings. The first edition of the adjuvant bibliography was compiled and published as a compendium of the literature up to 1986.[3] The second edition was expanded, revised, and issued as an appendix in *Adjuvants and Agrochemicals* by CRC Press in March, 1989.[10] Since 1986, a literature search of adjuvant formulations and their new application techniques was edited as another adjuvant bibliography.[5] A total of three searches in adjuvant literature located approximately 2000 references.

A survey of the bibliography of adjuvant literature over 60 years (1926 to 1989) revealed some interesting results, which will be discussed in this article.

II. BIBLIOGRAPHIC SURVEY

A. NUMBER OF PUBLICATIONS

In the early 1920s to 1940s, only one or two articles on the application of agro-adjuvants were published each year (Figure 1). As time progressed, the number of publications on adjuvants or related articles in various international journals increased at a rapid pace. In the 1960s, 90 to 150 articles were published during each 5-year period. During the 1970s and 1980s, more than 230 and 360 articles appeared in a 5-year interval, respectively. The number of publications continues to increase, as indicated by the 85 articles published in 1986.

It appears that the investigation of adjuvants has received more attention in the last 20 years. This can be seen in the comparison of 1966 and 1986, for example (Figure 1). There were more than three times as many publications in 1981 to 1986 than in 1961 to 1966. The reason may be associated with development of contact pesticides derived from cheap raw materials. In the early 1900s and 1920s, inorganic salts were used for controlling weeds, and kerosene and soap solutions were used for killing insects. Applied at high rates, these nonselective products did not need the aid of adjuvants to maximize their killing actions.

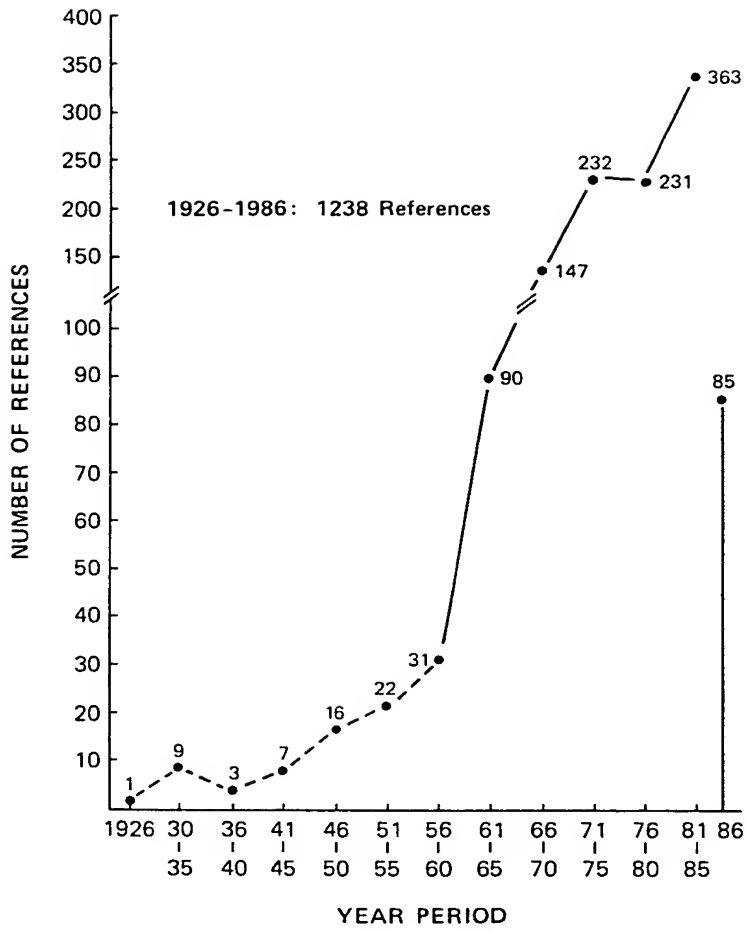

FIGURE 1. Number of publications of agro-adjuvants during the period of 1926 to 1986.

Although adjuvants had "wonderful", "magic" effects on the activity of herbicides,[4,6] the cost of applied herbicides in crop production was relatively low. Greater than recommended rates were applied to ensure better pest control. The sale of excess pesticides could also economically benefit the industry and encourage the development of more products. However, during the last 15 years, the economics of petroleum-based materials has changed rapidly and their components are no longer cheap. Therefore, research scientists and the chemical industry turned their efforts toward developing more efficient and cheaper adjuvants as well as pesticides. The efforts were fruitful. For example, the widespread use of a diuron mixture (Karmex) in the United States and the successful application of diclofop-methyl (Hoe-Grass, Hoelon) and sethoxydim (Poast, BAS 9152) mixed with adjuvants for weed control in Canada promoted a rapid increase in the use of adjuvants. European investigations have also demonstrated that the addition of appropriate adjuvants in the spray solution, prior to application, allowed a substantial reduction of the rates of pesticide ingredients required for effective pest control.[1] Thus, a number of adjuvant companies and dealers in Europe and North America have been established, and the sale of adjuvants has increased in recent years.

B. CLASSIFICATION OF BIBLIOGRAPHY

A total of 1490 references (from 1926 to May 1989) were derived into nine categories (Figure 2) based on their titles. It is interesting to note that over 70% of the articles dealt with physicochemistry and application techniques, from atomization and deposition size of formulations to agronomic aspects. Therefore, adjuvant effects on modifying the physical and chemical properties of the pesticide spray solutions are well documented. It is surprising that very few articles evaluated the "economic" impact of adjuvants added to herbicides or the impact on the "environment". Two cases may be worth mentioning here. Diclofop-methyl was initially registered at 1.1 kg/ha in Canada. Further testing including adjuvants met with success and the rate was reduced to 0.8 kg/ha and then to 0.6 kg/ha, with equal effectiveness in controlling grass weeds. Use of the reduced rate also increased the tolerance of barley to the herbicide.[2] A number of articles in the application category were found which investigated crop oil-based adjuvants with new grass killers, due to the economic advantage of their low application cost.

Adjuvants play an important role by inducing physiological responses directly or indirectly associated with phytotoxicity. For example, ethylene synthesis and phytotoxicity induced by agrichemicals (growth regulators, herbicides, surfactants, etc.) has been investigated in comparative detail.[7] However, as shown in Figure 2, metabolic study on surfactants in plants is limited.[8,9] Only one article related to the mode of action and physiological effect of oils on plant tissues was found, in which the author proposed that stomata penetration of oils was the principal path of entrance of oil into leaves.[11]

III. APPENDIX — SELECTED RELEVANT BIBLIOGRAPHY

The large number of entries in the bibliography of agro-adjuvants (about 2000 references in three sources) makes searching for needed references difficult and time consuming. Therefore, a selected relevant bibliography with 180 references in 7 classes and 21 subclasses as an appendix may provide a useful guide for finding references for research needs within a short time period.

The selection of references for a given category is intended to be a representative list from which readers may find more references in the reference section of each article. If an author published more than one article with a similar subject, only the most recent one was entered. Omission of the other articles does not indicate any bias in selection, nor does inclusion imply the best selection, as it is impossible to evaluate the content of each article within a limited time.

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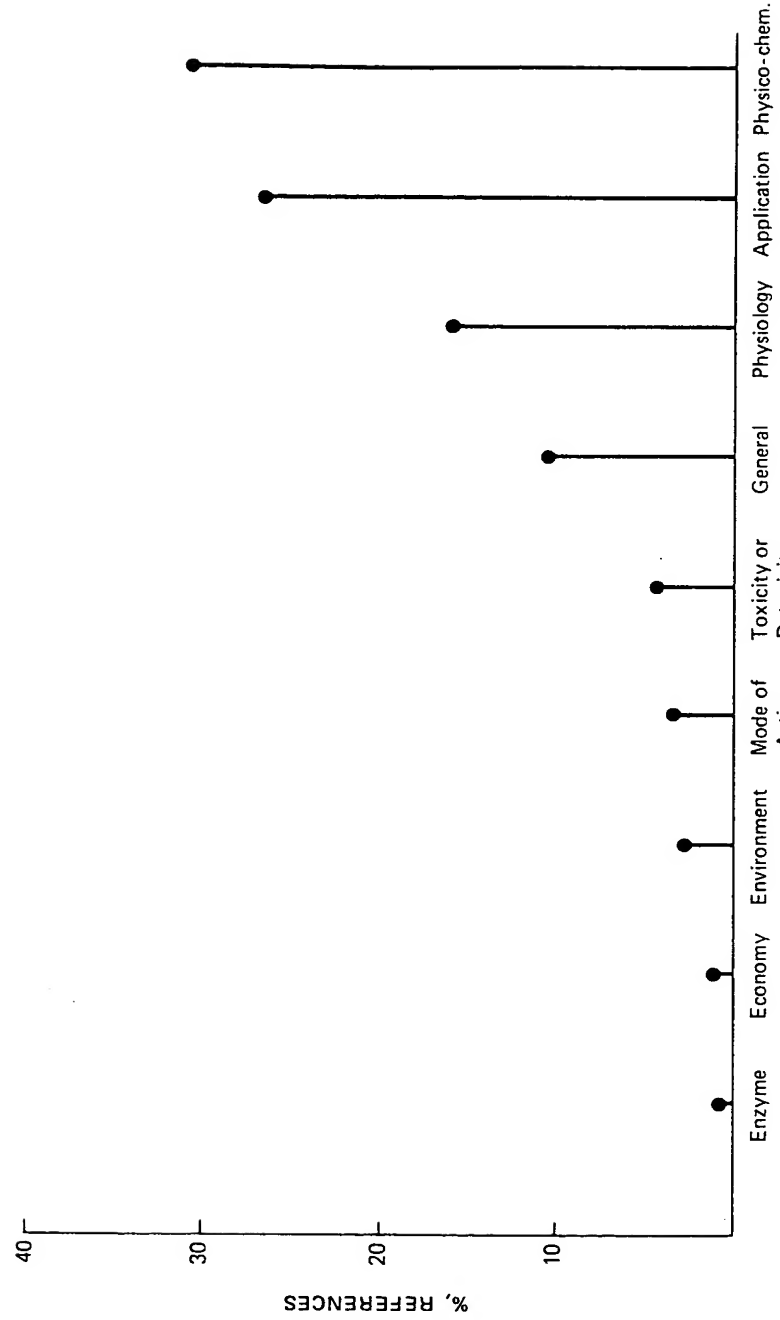

FIGURE 2. Distribution of 1490 classified publications of agro-adjuvants during the period of 1926 to (May) 1989.

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Chapter 2

ANALYSIS OF EFFECTS OF SURFACTANTS ON PERMEABILITY OF PLANT CUTICLES

Jörg Schönherr and Hubert Bauer

TABLE OF CONTENTS

ABSTRACT

The effects of surfactants on solute (2,4-D) and the water permeability of isolated cuticular membranes (CMs) were measured. In analyzing the data, surfactant effects on partition coefficients (K) and on diffusion coefficients in cuticles (D) are distinguished. Surfactants in donor solutions at concentrations above the critical micelle concentration (cmc) slow the penetration of solutes which are soluble in surfactant micelles. This effect is due to the reduced partition coefficients (cuticle/water) of solutes when the water contains surfactant micelles, and is called K-depression. Surfactants may also increase the mobility (D) of solute and water molecules in cuticles. This effect was studied using unilateral desorption of solutes from the outer surface (UDOS). In these experiments, 2,4-D sorbed in CMs must diffuse through a thin layer located at the outer surface of the CM. This skin of the CM is the layer that limits the velocity of desorption of 2,4-D. Rate constants of desorption using an inert phospholipid suspension and micellar surfactant solutions are compared. With surfactant solutions, rate constants of desorption were larger by a factor of up to about 40. Surfactant effects on rate constants of desorption increased with time and depended on the initial permeability of the CMs. This effect of surfactants is due to an increased mobility of 2,4-D in the limiting skin of the CM and requires the presence of surfactants in the CM. Our data and analysis show that activator surfactants must overcompensate K-depression by increasing diffusion coefficients in the limiting skin of the CM. Since activator surfactants must penetrate into cuticles, their effects depend on external concentrations, cuticle/water partition coefficients, and the diffusion coefficients of the surfactants. It is argued that activation of solute diffusion in CMs is due to increased segmental chain mobility in cutin and amorphous regions of soluble cuticular lipids (wax). Since water and solute permeation across cuticles are limited by the same barrier, the effects of surfactants on water and solute permeability are similar. Both the transpiration test and UDOS are suitable for screening surfactants and formulations for the activation of cuticular penetration of solutes.

I. INTRODUCTION

Surfactants are important constituents of pesticidal formulations. In conjunction with other adjuvants, they may function as spreaders, stickers, antifoamers, compatibility agents, or activators.[11] Activator adjuvants improve the efficacy of a formulation. It has been suggested that they function primarily by altering solubility relationships and therefore affect the ability of a pesticide to penetrate the cuticle.[24] This hypothesis puts the site of action of surfactants on the surface of the cuticle, and penetration of cuticles by surfactants would not be required for activation. In the meantime, it has been demonstrated that surfactants can penetrate cuticles rapidly and in substantial quantities.[22] Convincing evidence has been presented showing that surfactants penetrate the cuticles together with the active ingredients and that this copenetration might be one prerequisite for activation of the penetration of active ingredients.[7,10,22]

While it is well established that surfactants can speed up the penetration of active ingredients and water,[4,16] their mode of action at the level of the cuticle is not at all clear.[7] Diffusion across the cuticle is the rate-limiting step in foliar penetration from solutions.[19] If penetration occurs from more or less dry residues that remain on the cuticle after water and other volatile constituents of spray liquids have evaporated, the transition of active ingredients from the residue into the cuticle may add to the total resistance of the diffusive pathway. In addition to affecting coverage, retention, and the physical state of the residue on the surface of the cuticle, an activator surfactant could enhance penetration by changing

the structure and composition of cuticles. This could increase the diffusion coefficients in cuticles and therefore speed up penetration.

The complexity of solute uptake is one reason why the structure-activity relationships for surfactants in foliar penetration have remained obscure so far. Furthermore, in classical field and droplet experiments, many factors affecting uptake act and interact simultaneously. The contributions of individual factors to biological activity or to the amounts of active ingredients that have penetrated in a certain time cannot be separated. To overcome these difficulties, we have developed two test systems that specifically measure the mobility of water and solutes in plant cuticles. All other factors involved in foliar penetration from droplets and residues are absent in these tests.

II. MATERIALS AND METHODS

A. CUTICULAR MEMBRANES

Astomatous cuticles isolated enzymatically from mature green fruits of pepper (*Capsicum annuum* L. cv. Bell Boy), ripe fruits of egg plant (*Solanum melongena* L. cv. Black King), and the upper surfaces of mature leaves of bitter orange trees (*Citrus aurantium* L.) and pear trees (*Pyrus communis* L., cv. Bartlett) were used in the tests.[17] Isolated cuticles will be referred to as cuticular membranes (CMs). CMs from which waxes were extracted in a Soxhlet apparatus using chloroform will be referred to as polymer matrix membranes (MX membranes).

Fruits and leaves were taken from plants grown under controlled conditions in walk-in growth chambers (for details see Reference 5). Plants grew vigorously, remained healthy, and it was not necessary to use pesticides. The cuticles had therefore never been exposed to surfactants or pesticides prior to experimentation.

B. CHEMICALS

Diffusion across CMs and MX membranes was followed using radiolabeled solutes. (2,4-Dichlorophenoxy)-[2-^{14}C]acetic acid (2,4-D; specific activity 2.04 GBq/mmol) was obtained from Amersham Buchler (Braunschweig, Germany). Polydisperse [phenyl-^{3}H(N)]-Triton X-100 (specific activity 166.5 GBq/mg) was purchased from NEN (Dreieich, Germany). All other nonlabeled surfactants (Table 1) were polydisperse, with the exception of SDS, and were applied as unbuffered aquaeous solutions unless stated otherwise. 2,4-D was dissolved in 0.01 *M* potassium citrate buffer adjusted to pH 2.0.

A 1% (w/v) phospholipid suspension (soybean lecithin, Serva, Heidelberg, Germany) was used as a medium in the desorption studies. It was prepared by sonicating water and lecithin at 60°C for 15 min at full power using a Branson B-12 sonifier. NaN_3 (1 mmol) was added to the suspension to prevent the growth of microorganisms during the course of the desorption studies. This mixture was stable for months and will be referred to as phospholipid suspension (PLS).

Emulsions were prepared using low-viscosity paraffin oil (Merck 7174, Darmstadt, FRG) and the nonionic surfactants Brij 30, Brij 56, Myrj 45, and Tween 85. Paraffin oil (1.4 g) and surfactant (0.6 g) were mixed and diluted with water, resulting in a nominal coverage of 2 mg/cm^2 on the surfaces of the CMs.

C. PROCEDURES

The effect of surfactants on the water permeability of bitter orange and pear leaf CMs was studied using the transpiration test of Geyer and Schönherr.[4] Each CM was mounted on top of a transpiration chamber filled with water. The chambers were stored over dry silica gel (25 ± 1°C) and weighed repeatedly to monitor water loss through the CMs. When

TABLE 1
Surfactants Used for Experimentation

Trade name	Chemical name	nEO[a]	HLB[b]	Source[c]
SDS	Sodium dodecylsulfate	—	40.0	1
Triton X-100	*p*-(*t*-Octylphenyl)-ω-hydroxypoly(oxy-1,2-ethanediyl)	10	13.5	1
Triton X-35	*p*-(*t*-Octylphenyl)-ω-hydroxypoly(oxy-1,2-ethanediyl)	3	7.8	1
Brij 30	Dodecyl-ω-hydroxypoly(oxy-1,2-ethanediyl)	3	9.8	2
Brij 56	Hexadecyl-ω-hydroxypoly(oxy-1,2-ethanediyl)	9	12.9	2
Myrj 45	Polyoxyethylene stearic acid (monoester)	8	11.1	2
Tween 85	Polyoxyethylene sorbitol trioleate	20	11.0	1
Renex 36	Tridecyl-ω-hydroxypoly(oxy-1,2-ethanediyl)	6	11.6	2
Ethomeen C12	Bis(2-hydroxyethyl)cocoamine	1	10.2	3
Ethomeen C15	Polyoxyethylene cocoamine	5	17.9	3
Ethomeen T15	Polyoxyethylene tallowamine	5	14.9	3
Ethomeen T25	Polyoxyethylene tallowamine	15	19.3	3
Ethomeen S15	Polyoxyethylene oleylamine	5	14.6	3
Ethomeen S25	Polyoxyethylene oleylamine	15	19.2	3
Ethomeen HT15	Polyoxyethylene stearylamine	5	15.0	3
Ethomeen HT25	Polyoxyethylene stearylamine	15	—	3
Ethomeen HT60	Polyoxyethylene stearylamine	50	19.7	3

Note: SDS was reagent grade; all other surfactants were polydisperse and technical grade.

[a] Weighted mean of ethoxy groups per molecule of polydisperse surfactant.
[b] The hydrophile-lipophile balance (HLB) figures were taken from References 1, 6, 12, or from data sheets of the manufacturer.
[c] 1 = Serva, Heidelberg, Germany; 2 = Atlas Chemie, Essen, Germany; 3 = Akzo Chemie, Düren, Germany.

the water permeability of each CM had been established with good accuracy, aqueous surfactant solutions (50 μl) were applied to the morphological outer surface of the CM, resulting in a nominal coverage of 2 mg/cm². The effective coverage is only about half that amount, due to deposition of the surfactant on the apparatus.[4] When the water from the treating solutions had evaporated, weighing of the chambers was resumed as before. The ratio of the weight vs. time slopes after and prior to treatment with surfactant is a measure of the effectiveness of a surfactant. This ratio was calculated for each CM separately (paired observations). If a surfactant has no effect on water permeability, the ratio will be unity. The ratio will be larger than 1 if the surfactant increases water permeability. Deviations from unity were significant at the 95% level when the ratios were smaller than 0.7 or larger than 1.3. Means were calculated from effects observed for individual membranes. Standard deviations mainly represent variations in surfactant effects among CMs.

Water permeance (P) is the flow of water per unit area and driving force (liquid state), and was calculated using Equation 1:

$$P = (\Delta M/\Delta t)/(A \times \Delta C) \quad (1)$$

where the numerator represents the steady-state flow of water from the chambers (in kilograms per second), A is the surface area of the membrane exposed to water and silica gel (1.13×10^{-4} m²), and ΔC is the driving force for which 1000 kg/m³ were used, as the water concentration over dry silica gel is practically zero.

The effect of surfactants on solute permeability was studied using two different methods. Surfactant effects on the permeability of MX membranes was measured using the method of Kerler et al.[8] The MX membranes were inserted between the donor and receiver chambers

of the transport apparatus. The donor solutions containing radiolabeled 2,4-D and nonlabeled surfactant buffered at pH 2.0 faced the morphological inner surface of the membranes. A PLS served as receiver on the outer surface of the MX membranes. The apparatus was thermostated at 25 ± 1°C, and both donor and receiver solutions were stirred vigorously. The amount of 2,4-D that diffused across the membranes was measured as a function of time and, from the steady-state slope of a plot amount (M in dpm) diffused vs. time (t in s), the permeance (P) was calculated according to Equation 1, where A is the membrane area exposed to the solutions (0.38×10^{-4} m²) and ΔC is the concentration gradient (dpm per cubic meter) of nondissociated 2,4-D calculated from the pK_a (2.73) and the pH (2.0) of the donor. The concentration gradient across the membranes is solely determined by the concentration of the donor, as the 2,4-D concentration in the water is essentially zero in the receiver because the lipophilic 2,4-D is sorbed preferentially in the phospholipid aggregates of the PLS. This keeps the concentration of 2,4-D in water practically zero. In some early experiments, a 0.01 *M* borax buffer (pH 9.1) was used as receiver. At this pH, all 2,4-D in the receiver is ionized and the concentration of nonionized 2,4-D is also zero. By using the method of paired observations, the effectiveness of borax buffer and PLS as receiver media was compared and found to be identical (data not shown).

The donor concentration of 2,4-D was determined from aliquots of the donor solutions after the steady state had been reached. The amount of 2,4-D sorbed in the MX membranes was determined by carefully cutting out the areas of the membranes exposed to the solutions at the end of the experiments. They were blotted lightly to remove adhering solutions, and radioactivity was determined by scintillation counting (see below). The partition coefficient ($K_{mx/w}$) for the MX/water system was calculated from the mass of the MX (in kilograms), the radioactivity (in dpm) in the MX, and the equilibrium concentration of nonionized 2,4-D in the donor solution (dpm per kilogram) according to Equation 2:

$$K_{mx/w} = C_{mx}/C_{donor} \tag{2}$$

The permeance (P) as defined in Equation 1 is based on the concentration of nonionized 2,4-D in the donor solution. Since in these experiments the concentration of 2,4-D in the MX membranes was known, an alternative permeance denoted P* could be calculated using the concentration of 2,4-D in the MX membranes rather than the concentration of the donor solution. It follows from the definition of the partition coefficient (Equation 2) that the relationship between P and P* is simply

$$P^* = P/K_{mx/w} \tag{3}$$

P* is independent of the partition coefficient and therefore a measure of mobility (see below).

UDOS was used to study the effects of surfactants on the solute permeability of the CMs. The CMs were mounted between the sorption and desorption compartments of the apparatus shown in Figure 1. The desorption compartments were manufactured from conventional stainless steel (V2A) and the sorption compartments from Novorox FALC 22 3 (Krupp Südwestfalen AG, Siegen, Germany). This ferritic/austenitic steel is needed when solutions having a pH <3 are to be used in the sorption compartments. The contact areas of the sorption and desorption compartments were greased lightly (Hochvakuumsilikonfett, Wacker Chemie, München, Germany) to ensure a good seal. They were held together by three screws, with the CMs sandwiched in between. The inner surface of the CMs faced the sorption compartment.

The CMs were loaded with radiolabeled 2,4-D by adding a buffered solution (200 µl, pH 2.0) to the sorption compartment, which was left open so that the water could evaporate.

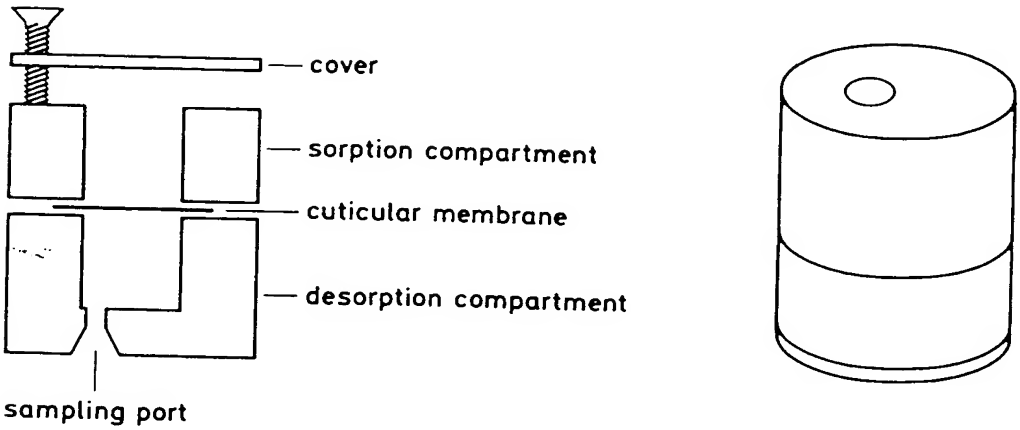

FIGURE 1. Apparatus used for unilateral desorption from the outer surface (UDOS).

If the evaporation of water is sufficiently slow, all 2,4-D will be sorbed in the CMs, and no residue will remain on the surface of the cuticle when all the water has evaporated. When the chambers were left standing without agitation on the top of the bench at ambient temperatures, sorption of 2,4-D in cuticles proceeded faster than evaporation of water. This was checked using ^{14}C-labeled 2,4-D and tritiated water. The $^{3}H/^{14}C$ ratio increased from 4 to 10 within 1 h and stayed at about that value until the water had evaporated after about 6 h (data not shown). As an alternative, loading can be performed with the sorption chambers covered. In this case, sorption solutions were removed after equilibrium had been established (24 h). This procedure was equally acceptable (data not shown), but large amounts of radiolabeled 2,4-D are wasted when the sorption solutions are discarded after equilibration.

It should be pointed out that rapid loading of cuticles with solutes works only through their inner surfaces, where diffusion coefficients are much higher than in the outer surfaces.[19]

When the water of the sorption solution had evaporated, the sorption compartment was closed with a stainless steel cover held in position by a small amount of silicon grease. This helps to avoid radioactive spillage in case a cuticle breaks during desorption, and it maintain 100% humidity in the sorption compartments. The chambers were then placed, with the sorption compartment facing down, into the holes of a thick aluminum block thermostated at 25 ± 1°C. This aluminum block was mounted on a rotary shaker that rocked the chambers horizontally during desorption at a frequency of about 60 cycles per minute. After temperature equilibration, 0.6 ml of PLS was added to each desorption chamber through the sampling port, using a syringe with a thin needle, and the shaker was activated. Desorption media were withdrawn quantitatively at 24 h intervals and were replaced by fresh PLS. After four successive samples had been taken, desorption was continued for an additional 4 d using 1% (w/v) aqueous surfactant solutions.

After termination of desorption, the covers were slipped off the sorption compartment, and the membrane areas exposed to the solutions were cut out carefully and placed in 5 ml of scintillation cocktail (Quickszint 212, Zinsser, Frankfurt am Main, Germany). After standing for 2 h, the radioactivity in the CMs was determined by scintillation counting (Packard CA 2000 scintillation counter). The high solubility of 2,4-D in this cocktail and the large mass of the cocktail relative to the mass of the cuticles ensured that all 2,4-D was quantitatively extracted by the cocktail, even from the thick pepper cuticles after 10 min (data not shown). The radioactivity in the desorption solutions was also determined after the addition of the cocktail (4 ml). All counting was performed at a 2 σ error of 1% and appropriate quench corrections were made.

The amount (M) of radioactivity desorbed from each CM at any time (t), denoted M_t, was obtained by summation of the radioactivities of successive samples. The total radioactivity initially contained in the CM (M_o) was calculated as the sum of the amounts desorbed plus the amount of radioactivity left in the CM after termination of desorption. Thus, the ratio M_t/M_o represents the relative amount desorbed and $1 - M_t/M_o$ is the relative amount of radioactivity in the CM at a given time t. These calculations were performed for each CM separately and the results plotted as $\ln (1 - M_t/M_o)$ vs. time. Each treatment (type and concentration of surfactant) was replicated 20 to 40 times.

The slope of the plot $\ln (1 - M_t/M_o)$ vs time is the first-order rate constant (k) of the desorption process. It can be related to P* as shown below.

It has been shown previously[18,19] that, structurally, cuticles are highly asymmetrical. They may be viewed as a laminate composed of a very thin outer layer (a "skin") having a low sorption capacity and a very low diffusion coefficient, and a thick inner layer with a high sorption capacity and a diffusion coefficient that is much higher than that in the skin. The inner compartment comprises most of the mass of the CM.

In UDOS, the inner sorption compartment is loaded with solute (2,4-D) and serves as the donor compartment. Diffusion of solute across the skin is rate limiting. The flow (F) is

$$F = \Delta M/\Delta t = V_{don}(\Delta C/\Delta t) \tag{4}$$

where V_{don} is the volume of the donor compartment of the cuticle. If ΔC changes with time, Equation 4 assumes the form

$$F = P^*A(C_{don} - C_{rec}) = -V_{don}(dC_{don}/dt) = V_{rec}(dC_{rec}/dt) \tag{5}$$

where A is the membrane area and P* is the permeance. The asterisk is necessary to prevent confusing it with the permeance P calculated using the solution concentrations as driving force. The subscripts don and rec refer to the donor and receiver compartments, respectively. The integral solution of Equation 5 is

$$-P^*A(1/V_{don} + 1/V_{rec})\, t = \ln(C_{don} - C_{rec})/C_0 \tag{6}$$

C_o is the donor concentration when $t = 0$. If the receiver concentration can be maintained at zero, Equation 6 reduces to

$$-(P^*A/V_{don})\, t = \ln C_{don}/C_0 = \ln(1 - M_t/M_0) \tag{7}$$

If $\ln (1 - M_t/M_o)$ is plotted against t, a straight line having slope (k) will be obtained and

$$k = -P^*A/V_{don} \tag{8}$$

Thus, from the first-order rate constant k, the permeance P* can be calculated if V_{don} is known. Since the skins of the cuticles have a finite but negligible thickness, we have assumed that V_{don} is equal to the total volume of the CM of area A. We have further assumed that the specific gravity of the CM is unity and calculated V_{don} simply from the mass of the CM. Since the specific gravity of cuticles is somewhat larger than unity, V_{don} of *Citrus* CMs will be underestimated by about 5%.[20] If the mass of the skin is 5% of the total mass of the CM, the two errors will cancel.

These assumptions are not crucial, since the surfactant effect is estimated by comparing the rate constants of desorption obtained with PLS with those obtained using surfactant

TABLE 2
Effects of Surfactants on Water Permeability of Cuticular Membranes from Leaves (*Citrus, Pyrus*) and Fruits (*Solanum, Capsicum*)

Surfactant	*Citrus*	*Pyrus*	*Solanum*	*Capsicum*
		Water Permeability in m/s		
P(CM) × 10^{10}	1.52	3.10	4.95	18.80
P(MX) × 10^{7}	1.56	0.98	1.19	1.28
P(MX)/P(CM)	1026	316	240	68
		Surfactant Effect on Water Permeability		
SDS	0.64—0.73	1.27—1.71	0.60—0.84	0.51—0.77
Triton X-100	2.46—3.16	5.63—9.17	1.24—1.70	2.30—2.88
Tween 85	2.78—3.95	6.54—7.96	1.93—2.53	2.22—3.22
Renex 36	4.49—7.13	20.14—42.38	2.56—3.58	2.48—3.92
Ethomeen T25	5.94—9.08	122.55—185.60	7.43—12.04	3.65—3.83
Brij 30	7.23—8.77	6.71—15.35	2.73—3.65	2.74—3.37

Note: Water permeances of CMs before treatment, P(CM), are means of 100 membranes; permeances of MX membranes, P(MX), are means of 10 membranes. Nominal surfactant coverage was 2 mg/cm². The surfactant effect is the ratio of water permeances after and prior to surfactant treatment. The surfactant effect was calculated for each CM individually and was averaged for 10 to 15 CMs. The 95% confidence intervals are given. They represent the variability in response of individual CMs.

solutions. The errors will cancel as long as the thickness of the skin is not affected by the surfactants. This is not likely to happen, and we have found no indications for such an effect of surfactants.

III. RESULTS

A. WATER PERMEABILITY

The effects of surfactants on the water permeability of the CMs depended on the type of surfactant and on the plant species (Table 2). SDS decreased the water permeability of *Citrus, Solanum, and Capsicum,* spp. CMs but slightly increased the permeability of some *Pyrus* CMs. All other surfactants increased the water permeability of the CMs of all four species, with Renex 36, Brij 30, and Ethomeen T25 being the most effective ones. The highest effects were always observed with CMs of pear leaves. Here, an increase in water permeability by factors of 122 to 185 was observed.

The effects of ethoxylated amines on the water permeability of *Citrus* CMs were very large and increased within each class (coco-, tallow-, oleyl-, and sterylamine) with increasing length of the oxyethylene chains. For a given degree of ethoxylation, the effect tended to increase with increasing length of the alkyl chains (Table 3). Ethomeen HT60 was almost ineffective.

Of the polyethylene gycols (PEGs) tested, the maximum effect was observed with PEG 400 (Table 4). PEG 4000 was ineffective.

When the time needed for evaporation of water from the treating solutions (*Citrus* CMs, 2 mg/cm² Renex 36) was varied, it was found that the effect was independent of the exposure time to micellar solutions in the range of 45 to 240 min. Longer time periods were not tried.

TABLE 3
Effects of Ethomeen Surfactants on Water Permeability of *Citrus* Leaf Cuticular Membranes

Surfactant	nEO	Alkyl chain	Effect	95% CI
Ethomeen C12	1	C12 (50), C14 (20)	5.53	4.67—6.39
Ethomeen C15	5	C12 (50), C14 (20)	6.30	5.67—6.93
Ethomeen T15	5	C16 (31), C18 (64)	6.62	5.88—7.36
Ethomeen T25	15	C16 (31), C18 (64)	7.92	6.77—9.07
Ethomeen S15	5	C16 (14), C18 (80)	7.24	6.61—7.87
Ethomeen S25	15	C16 (14), C18 (80)	7.64	6.39—8.89
Ethomeen HT15	5	C16 (31), C18 (64)	7.51	6.57—8.45
Ethomeen HT25	15	C16 (31), C18 (64)	10.08	9.18—10.98
Ethomeen HT60	50	C16 (31), C18 (64)	1.35	1.29—1.40

Note: Average ethylenoxide residues (nEO), average alkyl chain-length distribution, and relative amounts in percent (in parentheses) were obtained from the manufacturer. The C_{18} moiety of Ethomeen S15 and S25 is an oleyl rest. Water permeance of the CMs prior to surfactant treatment was 1.82×10^{-10} m/s. The effect of surfactant is the ratio of water permeance after and prior to surfactant treatment, calculated for each CM separately and averaged over 10 to 15 CMs per treatment. Nominal coverage was 2.0 mg/cm^2. CI, confidence interval.

TABLE 4
Effect of Polyethylene Glycols (PEGs) on Water Permeability of *Citrus* CM

Compound	nEO	Effect	95% CI
PEG 200	1—10(4)	1.73	1.63—1.83
PEG 400	3—20(9)	2.36	2.21—2.50
PEG 1000		1.61	1.36—1.86
PEG 4000		0.95	0.90—1.10

Note: Range of number of ethylene oxide (nEO) residues and most frequent homologue according to Engle et al.[3] Water permeance of the CMs prior to treatment was 1.98×10^{-10} m/s. The effect is the ratio of water permeance after and prior to treatment, with PEG calculated for each CM separately and averaged over 10 to 15 CMs per treatment. Nominal coverage was 0.2 mg/cm^2. CI, confidence interval.

The effects of surfactants applied to the outer surfaces of *Citrus* CMs on water permeability were not completely reversible (Table 5). When the surfactants were washed off the surfaces of the CMs, a significant residual effect on water permeability remained. The residual effect of Triton X-100 was barely significant.

Oil in water emulsions of liquid paraffin with nonionic surfactants increased water permeability more than the surfactants alone (Figure 2). This effect was not significant at the 95% level for all emulsions, but the tendency was the same in all cases. Paraffin oil alone did not affect the water permeability of *Citrus* CMs the (95% confidence interval of the effect was 1.14 to 1.40). The action of surfactants and paraffin oil is therefore cooperative.

TABLE 5
The Effect of Removal of Surface Residues of Surfactants on Water Permeability of *Citrus* CMs

Surfactant	Coverage (mg/cm^2)	Effect (95% CI)	Residual effect (95% CI)
Triton X-100	2.0	2.23—3.48	1.27—1.59
Renex 36	0.5	3.39—3.89	1.73—2.30
Brij 30	2.0	5.87—8.87	2.50—3.69

Note: Water permeance of the CMs prior to surfactant treatment was 2.05×10^{-10} m/s. The effect of surfactant is the ratio of water permeance after and prior to surfactant treatment, calculated for each CM separately and averaged over 10 to 15 CMs per treatment. After the effect had been determined, the surfaces of the CMs were washed extensively with water to remove the surface residues of surfactants, the chambers were filled with fresh water, and the permeance was determined again. The residual effect is the ratio of the permeances after washing the CMs over the permeances of untreated CMs. Nominal coverages given. CI, confidence interval.

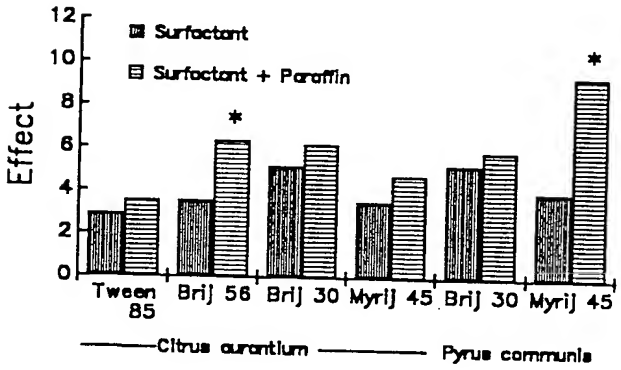

FIGURE 2. Effects of surfactants and surfactant/paraffin oil emulsions on water permeability of *Citrus* and pear leaf CMs. Nominal coverage was 0.6 mg/cm^2 for the surfactant treatment and 0.6 mg/cm^2 surfactant plus 1.4 mg/cm^2 paraffin oil for the emulsion. The effect is the ratio of water permeances after and prior to treatment. The effect of the emulsion is larger than the effect of the surfactant alone when marked with an asterisk (at the 95% level).

B. SURFACTANT EFFECTS ON 2,4-D PERMEABILITY OF POLYMER MATRIX MEMBRANES

Triton X-100 increased the 2,4-D permeance of green pepper MX membranes when present in the donor solution at a concentration of 0.01% (Figure 3A). Higher concentrations decreased 2,4-D permeance. At 25°C, the CMc of Triton X-100 is 0.019% (w/v)[23] This decrease in permeance at Triton X-100 concentrations above the cmc is caused mainly by the decrease of the MX/water ($K_{mx/w}$) partition coefficient of 2,4-D. Below the cmc, there was no effect of Triton X-100 on ($K_{mx/w}$) (Figure 3B). Triton X-100 slightly increased 2,4-D mobility (P*) in green pepper MX membranes when present at concentrations of 0.3% (Figure 3C).

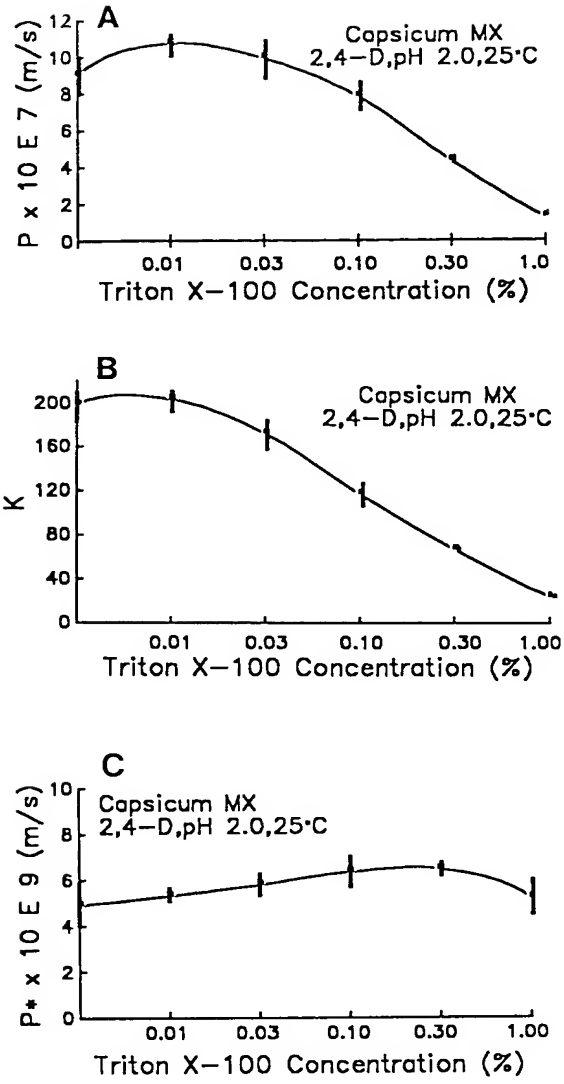

FIGURE 3. The effect of Triton X-100 on 2,4-D permeability of green pepper MX membranes. The surfactant was added to the donor.

Polydisperse ^{3}H-labeled Triton X-100 penetrated green pepper MX membranes. The permeance measured using a donor concentration of 0.01% (w/v) amounted to 1.40×10^{-7} m/s (confidence interval = 0.92 to 1.16×10^{-7} m/s), $K_{mx/w}$ was 108 (Cl = 95 to 121), and P* was 1.03×10^{-9} m/s (Cl = 0.91 to 1.13×10^{-9} m/s).

C. SURFACTANT EFFECTS ON 2,4-D PERMEABILITY OF CUTICULAR MEMBRANES

Surfactants in the donor in concentrations above the cmc will invariably result in reduced permeances when solutes interact with surfactant micelles. Micelles compete with cuticles as sorption compartments; the concentration of 2,4-D in the cuticles and the partition coef-

ficient decrease, and therefore P as well (Figure 3). As a consequence, surfactant effects on permeance can be analyzed only when their effects on the partition coefficients are measured simultaneously.

This problem does not arise when surfactants are added to the receiver solutions, provided the solute concentration in the receiver is maintained of practically zero. In this case, there will be no effect of surfactants on the driving force (the concentration gradient in the membrane) and any effects observed will be mobility effects.

To study the influence of surfactants on the mobility of 2,4-D in CMs, the cuticles were first loaded through the inner surfaces with 2,4-D in a sorption experiment. The 2,4-D was then desorbed through the outer surfaces, using an inert desorption medium. Initially, we used a borax buffer in which 2,4-D was fully ionized, the concentration of nonionized 2,4-D was therefore zero at all times. This, however, would work only with solutes that were weak acids. To make the test more general, a lecithin suspension was used as a desorption medium instead of borax buffer. The lecithin aggregates are large and do not penetrate the CM, but they serve as sorption compartments for lipophilic solutes. This keeps their concentration in the surrounding water practically zero. A comparison of the two desorption media using paired observations gave identical results. Phospholipid suspensions were ineffective in the transpiration test (data not shown).

UDOS of 2,4-D using a 1% phospholipid suspension (PLS) resulted in straight lines when results were plotted as a first-order rate process (Figure 4). The amounts and concentrations of 2,4-D sorbed in the CMs decreased exponentially with time. The slopes of the lines are the first-order rate constants of this process.

The effects of surfactants on this desorption process were investigated using the method of paired observations. The rate constant of desorption using PLS were established for each CM first. Then desorption was continued using micellar solutions of surfactants. The effects of the surfactants tested can be grouped as follows. (1) With SDS as description medium (Figure 5A), either no significant change in slopes was observed or the change was small and instantaneous. (2) With Ethomeen T25, the change in slopes was large, occurred rapidly, and the slopes remained linear until the experiment was terminated (Figure 5B). (3) Desorption with Triton X-100 resulted in curves having increasing slopes (Figure 5C). Desorption with Renex 36 at concentrations of 0.1% resulted in curves that resembled those in Figure 5C, but the time dependence of the slopes was more pronounced (Figure 5D).

All surfactants tested increased the rate constants of desorption of 2,4-D (Table 6). With most surfactants, the effect increased with time, and ranking of surfactants was done using the maximum effect. This is the ratio of the rate constant of desorption observed between the seventh and eighth day of experimentation and the rate constant between the first and fourth day using PLS. The mean maximum effect was very small with SDS, and increased in the other Triton X-100, Ethomeen T25, Brij 30, and Renex 36. Desorption with 1% Renex 36 increased the mean maximum rate constant of desorption by a factor of 16.64. The modes are in most cases smaller than the arithmetic means (negative excess), and the standard deviations, especially those of the means, are large.

The magnitude of the effect depended on the initial permeance (P*) as determined using PLS (Figure 6). The lower the initial P*, the larger the effect of the surfactant on the rate constant. The effects observed for individual CMs ranged from a 2- to a 40-fold increase in P* (Figures 6A to 6D). When the maximum effect of the surfactant was plotted against the reciprocal of the initial P*, a weak but significant correlation was obtained for all surfactants except the inert anionic SDS.

IV. DISCUSSION

Surfactants can markedly increase the permeability of plant cuticles for both water and solutes. How this is accomplished is the main topic of this section.

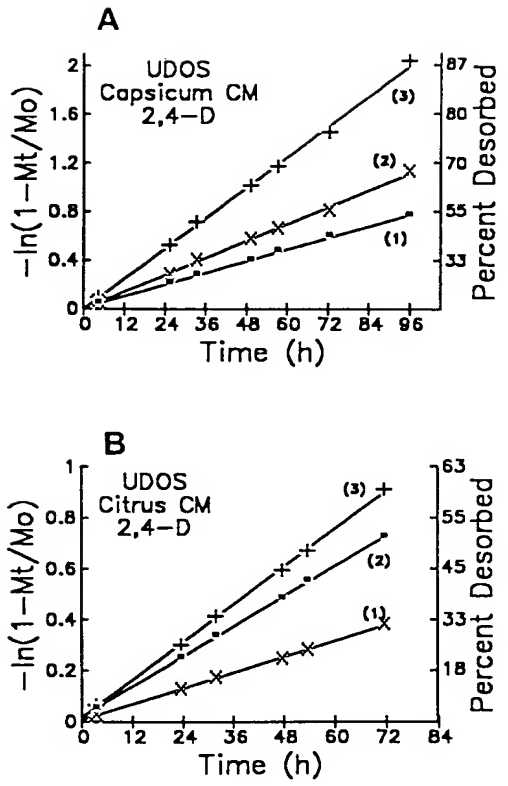

FIGURE 4. Unilateral desorption of 2,4-D from the outer surface of *Citrus* CMs using a 1% phospholipid suspension.

The barrier limiting the transport of water and solutes across CMs is located at the air/cuticle interface. It is a solid-state barrier composed of cutin and soluble cuticular lipids (waxes). The waxes are microcrystalline, and the crystallites represent excluded volumes for the diffusion of solutes and water because they are (on a short time scale) inaccessible. Diffusion is restricted to amorphous regions and to crystal/cutin interfaces.[14,19]

In homogeneous membranes, the permeability coefficient (p, the permeance of a membrane of unit thickness) is the product of the partition (K) and the diffusion coefficient (D) in the membrane:[2]

$$p = D \times K = P^* \times \ell \times K \tag{9}$$

Thus, there are three ways in which surfactants can affect the permeability of CMs to water and solutes: they can affect either K, D, or both. When permeance is calculated using the solute concentration in the cuticle as the driving force, $P^* \ell$ is equivalent to D (Equations 3 and 9). The sites of action can be cutin, amorphous waxes, or the cutin/wax interfaces

Surfactant effects on cutin or the polymer matrix are easy to understand. Surfactants in the donor at concentrations above the cmc will reduce the cuticle/water ($K_{mx/w}$) partition coefficient for the solute, and hence will reduce the permeance proportionately (Figure 3). This K-depression is due to solubilization of the solute molecules in micelles, and for a given surfactant it increases with increasing number of micelles, that is, with increasing concentration of surfactant. The K-depression also depends on the solubilization capacity

TABLE 6
Effects of Surfactants on Rate Constants of Desorption (UDOS) of 2,4-D from *Citrus* Cuticular Membranes

Surfactant	n	Initial P* ± SD ($\times 10^{12}$ m/s)	Effect	
			Mean ± SD	Median ± SD
SDS	25	2.99 ± 0.85	1.73 ± 0.45	1.67 ± 0.09
Triton X-100	38	3.36 ± 1.31	5.26 ± 2.57	4.41 ± 0.42
Ethomeen T25	30	2.00 ± 0.73	10.17 ± 4.59	10.24 ± 0.85
Brij 30	22	2.13 ± 1.18	13.47 ± 5.22	12.42 ± 1.11
Renex 36	29	2.76 ± 2.12	16.64 ± 9.67	16.50 ± 1.60

Note: The initial P* is the permeance measured using PLS. It was calculated from the rate constant of desorption according to Equation 8. The effect is the ratio of the rate constants measured during the last desorption period (day 7 to day 8) with surfactant to the rate constant measured using PLS. It represents the maximum increase in rate constant or permeance observed. The number of CMs studied (n) and standard deviation (SD) are given. Surfactant concentration was 1% (w/v).

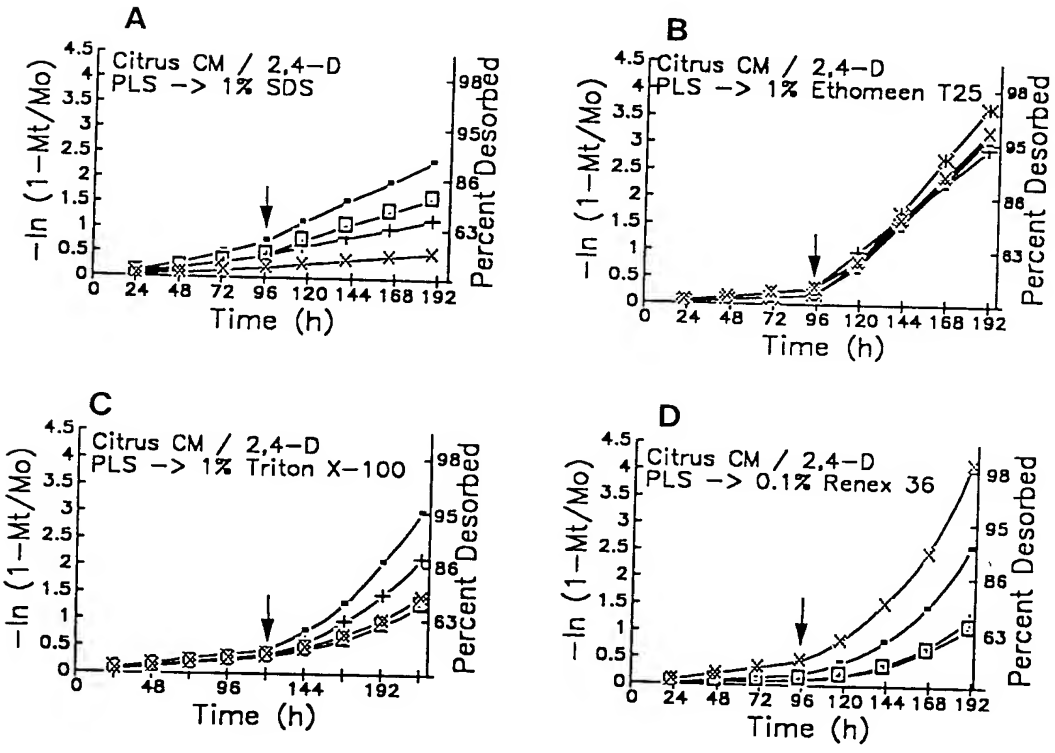

FIGURE 5. Unilateral desorption of 2,4-D from the outer surface of *Citrus* CMs. Up to 96 h, the desorption medium was a 1% phospholipid suspension (PLS); 1% surfactant solutions were used between 96 and 192 h.

of a given surfactant for a given solute.[9] For polar solutes, which are not solubilized in micelles, the K-depression will be absent, but with lipophilic solutes, the K-depression will invariably slow penetration by decreasing P. The magnitude of this K-depression will depend on the type of solute, type of surfactant, and its concentration.[9]

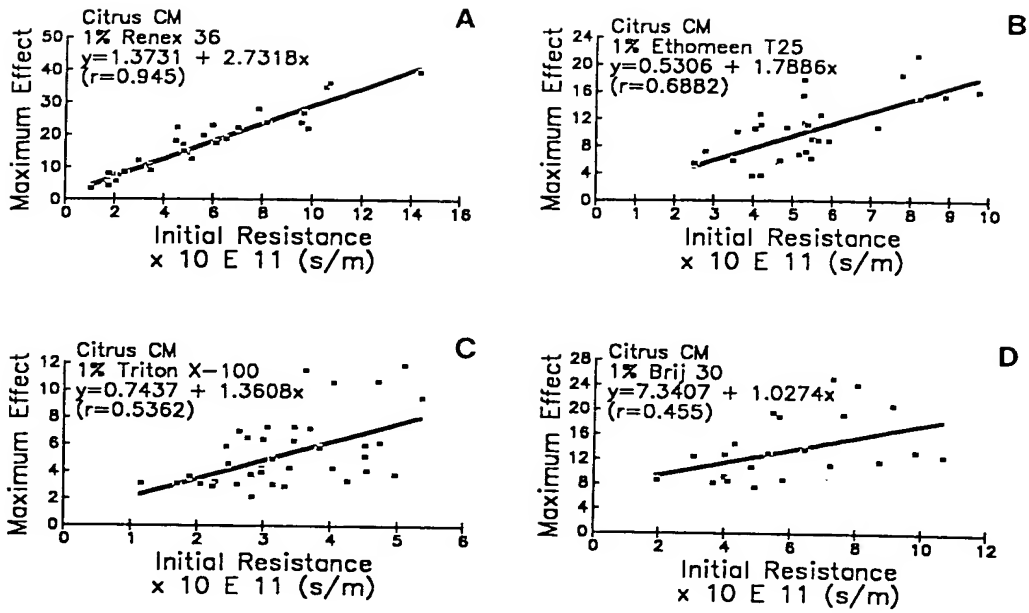

FIGURE 6. Correlation of initial 2,4-D resistance of *Citrus* CMs with the maximum effects of surfactants on rate constants of desorption in UDOS. Initial resistance is the reciprocal of initial permeance (P*) determined using 1% PLS. The effect is the ratio of maximum rate constants measured using surfactant solutions for desorption over the rate constants observed using PLS.

Depending on surfactant concentration (Figure 3), a small effect on solute mobility in the polymer matrix can be observed. This slight increase in P* indicates an increase in polymer chain mobility. However, 2,4-D permeance was much more affected by the K-depression than by an increase in P* as long as the surfactant concentration was above the cmc. Below the cmc, there was no surfactant effect on $K_{mx/w}$, and a slight increase in P* was the sole effect of Triton X-100 (Figure 3A).

A surfactant effect on P* requires that the surfactant be present in the polymer matrix. This poses no serious problems, since nonionic surfactants are soluble in cuticles.[21] Our data for green pepper MX membranes also show a good solubility of Triton X-100 ($K_{mx/w}$ = 108). In equilibrium with a Triton X-100 solution of 0.01%, the MX contained 10.8 g of Triton X-100 per kilogram of MX. The 2,4-D concentration in the MX of experiments shown in Figure 2 and Table 6 amounted to only 7×10^{-3} g of 2,4-D per kilogram of MX. This means that for every 2,4-D molecule in the MX there were about 550 molecules of Triton X-100.

Analyzing surfactant effects on the permeability of CMs is more difficult. The analysis must again center around the surfactant effects on K and P*, but interactions between surfactants and waxes must also be considered. Using CMs from *Citrus* and pear leaves, Riederer and Schönherr[16] have shown that aliphatic constituents of cuticular waxes (alcohols, alkylesters, and alkanes) were neither dissolved nor solubilized by copious quantities (2 mg/cm^2) of SDS, Triton X-100, Brij 30, Tween 85, Renex 36, and Ethomeen T25. Hence, the effects of these surfactants on water and solute permeability demonstrated in this study cannot be attributed to the loss of these types of soluble cuticular lipids. In the next analysis, we shall discuss surfactant effects on water and solute permeabilities separately, because experimental conditions and surfactant action in the transpiration test and in UDOS are not completely comparable.

The fact that UDOS resulted in straight lines when ln $(1 - M_t/M_o)$ was plotted against time (Figure 3) shows that the assumptions inherent in Equations 4 to 8 are valid. 2,4-D sorbed in the inner regions of the CM diffuses across the rate-limiting skin into the receiver solutions, where it is trapped in either the PLS or the surfactant micelles. This linearity is evidence that the diffusion coefficient in the limiting skin is much lower than in the sorption compartment. Thus, diffusion of 2,4-D in the inner sorption compartment is not rate limiting. It is diffusion in the skin that is rate limiting. The rate constants obtained using UDOS therefore represent transport properties of the skin rather than those of the entire CM.

UDOS has a number of properties that make it well suited to quantifying and analyzing surfactant effects on solute permeation in the limiting skin of cuticles. (1) The driving force is the solute concentration in the cuticle rather than the concentration gradient between donor and receiver solutions. This eliminates the effect of surfactants on the partition coefficient (K-depression). (2) The effects of surfactants are measured for each CM individually because UDOS uses the method of paired observations: each CM is control (desorption with PLS) and treatment (desorption with surfactant). This makes the test very sensitive, as it eliminates the effects of natural variability of permeabilities among CMs. It also provides the possibility of correlating surfactant effects with the permeance of untreated CMs (initial permeance), as shown in Figure 6. (3) Surfactant effects can be quantified using rate constants which are obtained without making any assumptions. (4) Even the assumptions needed to calculate P* do not cause problems when permeances of different solutes or effects of different surfactants on permeance for a given type of cuticle are compared. In all of these cases, the assumptions concerning the size of V_{don} will cancel. (5) For a given type of CM, P* is equivalent to D (Equation 9), it is essentially a mobility, and any surfactant effect observed with UDOS in an effect on mobility.

All surfactants tested using UDOS had a large effect on the mobility of 2,4-D in *Citrus* CMs (Table 6). SDS was the only exception, and its small effect can be considered negligible. Effects that are rate constants increased with time (Figure 5) until the experiments were terminated. It was not possible to continue desorption with surfactants because there was very little 2,4-D left in the CMs after 4 d of desorption with surfactant solutions (Figure 5). With these surfactants, maximum effects could not be determined. This indicates that surfactant penetration into the cuticles was very slow, and equilibrium between desorption media and cuticles was not obtained in 4 d. The maximum effect of Ethomeen T25 was obtained rapidly, after only 1 to 2 d (Figure 5).

Surfactant effects were inversely related to the initial resistance (1/P*) of the CMs (Figure 6). The mobility of 2,4-D in CMs having a low initial permeance was much more affected by surfactants than in CMs having a high initial permeance. It remains to be seen if surfactant effects on the solute permeances of cuticles from other species having much higher initial permeances than *Citrus* CMs will also be smaller.

Since surfactant effects were dependent on time and initial permeance, it is difficult to rank surfactants according to activity. If one uses the maximum effects observed (which are not identical to the maximum effects possible, except for Ethomeen T25) as a criterion, the sequence of increasing activity was Triton X-100, Ethomeen T25, Brij 30, and Renex 36. HLB values of these surfactants ranged from 9.8 to 19.3 (Table 1).

The time dependence of the surfactant effect in UDOS is evidence that surfactants must penetrate the skin of the CM to be effective. For an effect located at the very surface of the CM, an immediate response would be expected. Penetration of surfactant monomers into the CM seems to be a slow process since, with most surfactants, effects increased from the first to the fourth day. This is not surprising, since diffusion coefficients of solutes in cuticles depend heavily on molar volumes[19] and ethoxylated surfactants are rather large molecules. This effect of surfactant size on permeance can be seen by comparing the permeances of

2,4-D and Triton X-100 in green pepper MX. The permeance of 2,4-D was 5.4 and P* was 4.8 times larger than for polydisperse Triton X-100. This difference is likely to be even larger in CMs.

The relationship between surfactant effects on the mobility of 2,4-D in the skin of CMs and the concentration of the surfactant there cannot be deduced from our data. Before our results can be fully understood, sorption in and permeance of surfactants across CMs must be studied in relation to surfactant properties.

Our results and the above discussion show that at least four parameters are needed to quantitatively describe the activity of surfactants on solute mobility in cuticles. (1) The effect depends on the concentration of the surfactant in the cuticle. The cuticle/water partition coefficient together with concentration of surfactant monomers in the aqueous phase quantitatively account for this effect. (2) The surfactant must enter the cuticle, and if permeation occurs through the outer skin (as in actual practice), this will be a slow process determined by the diffusion coefficient in the cuticle (or by P*) and by the driving force. (3) The interactions between surfactants, cutin, and amorphous waxes that lead to increased mobility of solutes must be suitably quantified, possibly by a parameter related to segmental chain mobility in cutin and in amorphous waxes. (4) If surfactant effects depend on surfactant-solute interactions and not only on surfactant-cuticle interactions, this effect will have to be accounted for by yet another parameter. This argument will not be discussed further, since all surfactants were polydisperse. We do not know which homologues were active and to what degree. To answer these questions and to analyze structure-activity relationships in UDOS, the effects of monodisperse surfactants in UDOS must be studied. Since water and solutes have to overcome the same barrier in cuticles, one might expect that the surfactant effects on solute and water permeability are the same, providing the surfactants affect water permeability solely by affecting the mobility of water in the skin.

The transpiration test was initially designed to screen surfactants for effects on water permeability.[4] The aim was to distinguish between active and inactive surfactants. The magnitude of the effect was less important. In the present study, we had the same goal, and this is why we used very high coverages (2 mg/cm^2) for screening surfactants.

Ethoxylated surfactants are sorbed in cuticles.[21] The polyoxyethylene chains of sorbed surfactants are probably hydrated. This increases the water concentration in the CMs, and the effect of surfactants is therefore a mixed effect: both the mobility (D) and the partition coefficient of water in the cuticles are likely to be increased by active surfactants. These two effects cannot be separated unless the amounts of surfactant sorbed in the CM and the amount of water associated with the polyoxyethylene chains are known. This is not the case at the present time, and the surfactant effects on water permeability shown in Tables 2 through 4 cannot be fully analyzed.

All active surfactants are technical products and polydisperse. The effects observed are averages of the effects of individual homologues. These homologues differ in ethoxylation, and thus in polarity and in molecular weight. Their partition and diffusion coefficients in cuticles will differ. It is therefore no meaningful to attempt to correlate surfactant structure to activity in the transpiration test using our data obtained with polydisperse surfactants. For this type of analysis, the effects of monodisperse surfactants on the water permeability of CMs are needed. A few general points can be made, however.

1. Ethoxylation seems to be necessary for activity. Even PEGs were active (Table 4). The activity seems to depend on the mole ratio of hydrophobe and polyoxyethylene chains (Table 3). However, there was no clear relationship between HLB and the activity of surfactants. Very active surfactants had HLB values ranging from 10 (Brij 30) to almost 20 (Ethomeens). Excessive size (Ethomeen HT 60, PEG 4000) seems

to prevent activity, possibly because these very large molecules cannot penetrate the cuticles. SDS is small, but negatively charged, very polar, and therefore not sorbed in the CMs. It is inactive because it cannot enter the CMs.

2. Surfactant effects on water permeability were only partially reversible (Table 5). Since cuticular waxes are not dissolved or solubilized by these surfactants,[16] this residual effect indicates that the surfactants had not been washed out completely. In fact, the surfactants were detected in cuticular waxes extracted from cuticles that had been treated similarly.[13]
3. Surfactant effects were larger with UDOS than in the transpiration test. 2,4-D molecules are much larger than water molecules and they experience much more hindrance in the CMs than water. In fact, the permeance of 2,4-D in *Citrus* CMs is about 6×10^{-10} m/s (calculated as $P^* \times K$, using the data of Table 6 and a K of 300),[15] while water permeance is 1.5×10^{-10} m/s (Table 2). Is therefore seems that larger molecules benefit much more from surfactant effects than smaller ones. This agrees with the observation that 2,4-D mobility in CMs was much more affected by surfactants when the initial permeance was low (Figure 6).
4. Differences in the magnitudes of surfactant effects between the two tests may also be due to different states and concentrations of surfactants in the two types of experiments. In the transpiration test, the surfactants occur as a highly concentrated phase on the surface of the cuticles. The proximity of the dry silica gel and the low water permeances of most cuticles will result in very low water content of the neat surfactant phase. With *Citrus* and pear leaf CMs, the surfactant phase was thicker than the CMs themselves, which had an average mass of only 0.25 mg/cm^2. In UDOS, surfactants were used as diluted micellar solutions. This difference will lead to different (but unknown) concentrations of surfactants in the cuticles and hence to different effects.
5. The sequence of effectiveness with *Citrus* CMs was similar in both types of tests: SDS was almost ineffective, Triton X-100 had the lowest effect, and Brij 30, Ethomeen T25, and Renex 36 were the most effective. It appears that the hydration water of surfactants sorbed in the cuticles did not contribute greatly to the total effect of surfactants in the transpiration test. Thus, both tests are suitable for screening surfactants for activity. They may also be used to test emulsions (Figure 2) or complete formulations.

We shall employ the tests in the future to investigate structure-activity relationships using monodisperse surfactants. To the manufacturers of pesticides, the tests can be useful tools for screening formulations for their effectiveness in increasing the mobility of active ingredients in cuticles. This will help to better understand the complex results obtained in field trials or with droplet experiments. The outcomes of these experiments depend on many factors, such as wetting, spreading, retention, coverage, state of surface residue, volatility of active ingredients, partitioning of active ingredients, and diffusion across the cuticles. All these factors are affected by surfactants. With UDOS, the effects of surfactants on the mobility of active ingredients in cuticles can be measured specifically without interference by the factors mentioned above.

ACKNOWLEDGMENT

This chapter was supported in part by the Deutsche Forschungsgemeinschaft.

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Chapter 3

RELATIONSHIP BETWEEN SURFACTANT PROPERTIES AND WETTABILITY OF RICE LEAF SURFACES FOR SEVERAL NONIONIC SURFACTANTS

B. J. Chung and Y. W. Kwon

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ABSTRACT

The wettability of several important nonionic surfactants on rice leaf surfaces was examined at the active tillering, and heading stages for eight rice varieties differing widely in characteristics of leaf morphology. Surfactants were chosen from homologous series of polyoxyethylene (POE) nonylphenyl ethers (NP), POE octylphenyl ethers (OP), POE sorbitans (sorbitans), and polystyrenated phenols (SP) having different ethylene oxide (EO) content and hydrophile-lipophile balance (HLB) value.

Scanning electron micrographs showed noticeable differences in the fine morphology of the leaf surface, particularly epicuticular wax deposits, among the varieties. The glabrous varieties had a denser wax coverage than the long-hairy and the pubescent, while the water wettable had the least wax coverage.

The contact angle on the leaf surface increased with an increase in the HLB value and logarithmically with an increase in surface tension. At HLB values of 12 to 13, the surface tension of the surfactants and the contact angle on the leaf surfaces showed the lowest value and adhesional force the highest value. Comparing surfactant groups, NPs and OPs had lower surface tension and contact angle, and higher adhesional force than sorbitans and SPs. Contact angles were lower, but adhesional forces were higher at the heading stage than at the tillering stage. Growth stage-dependent difference in adhesional force at a surface tension of 35 dyn/cm was not observed with NPs and OPs having HLB values of 12 to 13. Adhesional forces were related to contact angles ($r = 0.948^{**}$) for all eight varieties at both growth stages.

For the eight varieties, contact angle increased more on water-wettable leaf surfaces than on glabrous, long-hairy, or pubescent leaf surfaces, but adhesional forces decreased less as surface tension increased. These results suggest that the wettability of rice leaf surfaces varies mainly with the degree of wax coverage of the rice varieties and with the particular growth stage.

From the above, it is concluded that adhesional force is a better criterion than contact angle in selecting for increased wettability of rice leaves.

I. INTRODUCTION

In order to control pests effectively, pesticides sprayed onto plants must be taken up and subsequently translocated to the active site.[18] Uptake into plants is dependent on spray retention and spreading on plant surfaces.[17] Effective spray retention is known to be enhanced by good wetting of plant surfaces.[2,3]

Epicuticular waxes, trichomes, various hairs, and other protuberances of the leaf surface are known to act as barriers to pesticide uptake. The addition of surfactant is necessary to provide adequate wetting.[4,6,9,12]

Surfactants have often been classified according to their hydrophile-lipophile balance (HLB), moles of ethylene oxide (EO), and/or surface tension of dilute solution. In terms of the wettability of a surfactant solution, evaluations have been made according to the contact angle they form on target surfaces.[1,4,5,8,11] Since these criteria, but not the adhesional force, have been used rather exclusively to study the relationship between surfactants and target surfaces, it may be valuable to examine the significance of the adhesional force (surface tension multiplied by the cosine value of the contact angle) of surfactant solutions on intact rice (*Oryza sativa* L.) leaf surfaces.

The present study was carried out to establish guidelines for the proper selection of nonionic surfactants in the formulation of pesticides for rice culture. We were interested in elucidating the relationship between surfactant properties and the wettability of rice leaf

TABLE 1
Rice Varieties Used in the Present Study

Variety	Source	Leaf surface property	Type
M 101	Cultivar of U.S.A.	Glabrous	Japonica
wx 817	wx 817-1-65-2-1	Glabrous	Tongil[a]
LK 2-7	LK2-7-12-1-1	Long-hairy	Japonica
HP 914	HP 914-3-2-1-1	Long-hairy	Tongil
Chucheong	Cultivar of Korea	Pubescent	Japonica
Cheongcheong	Cultivar of Korea	Pubescent	Tongil
HP 854	HP 854-8-1-2-8-1-1	Water wettable	Japonica
wx 139	wx 139-3-64-2-3-1-1	Water wettable	Tongil

[a] Variety bred through crosses of Japonica and Indica varieties.

surfaces for several important nonionic surfactants. Rice varieties differing widely in gross leaf morphology and genetics (so-called glabrous, long-hairy, pubescent, or water-wettable leaf properties for Japonica and Indica × Japonica types) were studied. The wettability of rice leaf surfaces was examined at the active tillering and heading stages with surfactants having different HLB and EO molar values.

II. MATERIALS AND METHODS

A. PREPARATION OF RICE LEAVES

The eight rice varieties used herein differed widely in the gross morphology of their leaf surfaces and were obtained from the Crop Breeding Laboratory of the Agronomy Department, Seoul National University and Crop Experiment Station of Korea. Table 1 shows the source, leaf surface property, and varietal type of the varieties used. They were cultured in ordinary paddy fields at the Hannong Central Research Institute.

B. SCANNING ELECTRON MICROSCOPY (SEM)

Rice leaf sections of 5 cm were taken from the center of a flag leaf. The sections were dehydrated using the Gabriel method[5,7] and then dried by the critical point drying method.[7] The samples were coated by the U-520 (Polaron, England) at 18 mA, 10^{-2} to 10^{-4} mbar for 120 s. The Hitachi Model S-570 SEM was used to observe specimens at an accelerating potential of 24 kV. SEM photographs were taken for both sides of the same leaves at a magnification of 500 and 5000.

C. PREPARATION OF SURFACTANT SOLUTIONS

The nonionic surfactants used in this study are listed in Table 2. All surface tension and contact angle measurements were made using a 0.1% (w/v) solution of the surfactants.

D. MEASUREMENT OF SURFACE TENSION

The surface tension of each solution was measured by counting droplets with a 5-ml droplet-counting apparatus and then calculated by the formula:[10]

$$rl = \frac{no}{n} \times rw$$

where rl is the surface tension of a surfactant solution; no, the number of droplets of distilled water; n, the number of droplets of the surfactant solution; and rw, the surface tension of distilled water at 25°C. Each measurement was replicated three times.

TABLE 2
Physicochemical Characteristics of the Nonionic Surfactants Used

Product name	Chemical description	Mol No. of EO	HLB	Supplier
Koremul NP-4	POE nonylphenyl ether	4	8.9	HNCI
Koremul NP-6		6	10.9	
Koremul NP-8		8	12.3	
Koremul NP-10		10	13.3	
Koremul NP-16		16	15.0	
Koremul NP-20		20	16.0	
Koremul NP-30		30	17.1	
Triton X-45	POE octylphenyl ether	5	10.4	R & H
Koremul OP-8		8	12.4	HNCI
Triton X-100		9—10	13.5	R & H
Triton X-102		12—13	14.6	R & H
Triton X-305		30	17.3	R & H
Tween 85	POE sorbitan triolate	20	11.0	Reagent
Tween 21	POE sorbitan monolaurate	4	13.3	
Tween 80	POE sorbitan monooleate	20	15.0	
Tween 20	POE sorbitan monolaurate	20	16.7	
SP 310F	Polystyrenated phenols	—	13.1	HNCI
SP 311F		—	13.3	
SP 309F		—	15.8	
Triton CS-7	Mixture of Triton X-114 (36) and Triton GR (5 *M*, 24%)		12.2	HNC

Note: EO, ethylene oxide; HLB, hydrophile-lipophile balance; HNCI, Hannong Chemical, Inc.; HNC, Hannong Corporation; R & H, Rohm & Haas.

E. MEASUREMENT OF CONTACT ANGLE

A 2-μl droplet of surfactant solution was applied to intact fresh rice-leaf blades mounted on glass slides with double-stick tape, using a micropipette fitted with a syringe needle (10 μl, Hamilton). Pictures of the droplet on the intact leaf surface were taken (under reflected light) 2 min after each application with a Nikon FG 54-mm camera equipped with a close-up lens. The contact angle of the droplets was determined by projection/magnification on a screen. Each measurement was replicated three times.

Calculation of adhesional force

Adhesional force (Wa) was calculated by the formula:[10]

$$Wa = rl \times \cos_{\theta}$$

where rl is the surface tension of the surfactant solution (0.1% w/v), which equals the contact angle_{θ} of the droplet of surfactant solution on the rice leaf surface.

III. RESULTS AND DISCUSSION

A. FINE MORPHOLOGY OF LEAF SURFACE AND EPICUTICULAR WAXES OF RICE VARIETIES

SEM photographs showed noticeable differences in the fine morphology, especially of epicuticular wax deposits, of the leaf surface in the varieties examined. In the glabrous rice varieties, bicellular microhairs and small papillae protruded over the entire leaf surface (Figure 1). Long hairs and unicellular microhairs were seen in the long-hairy rice varieties (Figure 2). In the pubescent rice varieties, unicellular and bicellular microhairs, small papillae, large inflated papillae, and trichomes protruded over the leaf surface (Figure 3).

Bicellular microhairs, small papillae, and large inflated papillae were seen in the water-wettable rice varieties (Figure 4). The epicuticular wax deposit was platelet-shaped and deposit on the cuticle layer. Similar observations were previously made by Takeoka at el.[19] The glabrous varieties had a more dense coverage of epicuticular waxes than the long-hairy and pubescent varieties, while the water-wettable rice varieties had the least coverage. Epicuticular waxes are an important barrier to the wetting of the leaf surface, and in this study wettability seemed to be dependent on the degree of wax coverage on the leaf surface.

B. RELATIONSHIP BETWEEN THE SURFACTANT PROPERTIES AND WETTABILITY OF NONIONIC SURFACTANTS

Low surface tensions were obtained for surfactants in the range of 12 to 14 HLB values, regardless of the number of moles of EO and the surfactant groups (Figure 5). It is well known that interfacial tension between the spray solution and plant surface must be reduced to aid wetting and the penetration of pesticides into leaves,[3,8,13-15,17] indicating that reduced surface tension increases the wettability of spray solutions. The HLB values of nonionic surfactants commonly used in agrochemical formulations have been reported to be in the 12 to 15 range.[11] Our results seem to justify this practice.

With respect to surfactant groups, NPs and OPs had lower surface tension than the sorbitans and polystyrenated phenols. Our results show that NPs and OPs having HLB values in the 12 to 14 range reduce surface tension more effectively than other surfactants.

Contact angles were low for surfactants having HLB values of 10 to 13. They were also lower at the heading stage than at the tillering stage (Figure 6). NP-8 and NP-10, and X-45 and OP-8, having low contact angles and surface tension among nonionic surfactants, all had HLB values of 12 to 14 (Figure 6).

As shown in Figure 7, contact angle increased logarithmically with an increase in surface tension. At the heading stage, a significant relationship between contact angle and surface tension was obtained: Y (contact angle) = 160 ln × −560.5 (r = 0.848**). Likewise, the relationship at the tillering stage was Y = 160.1 ln × −528.6 (r = 0.831**).

Exceptionally, NP-4 and NP-6, marked by circles in Figure 7, had a low contact angle even though they had high surface tension. This may be due to the fact that these surfactants have poor water solubility and better compatibility with the intact leaf surface due to their lipophilic properties.

Adhesional forces generally decreased with an increase in the HLB value, showing high values in the 12 to 14 HLB range (Figure 8). Among surfactants having high adhesional force and low surface tension, NP-8 and NP-10, and OP-8 and X-100 all have HLB values between 12 and 14.

As shown in Figure 9, adhesional force decreased negatively with an increase in surface tension. At the heading stage, a significant relationship between adhesional force and surface tension was obtained: Y (adhesional force) = $3596.7X^{-1} - 71.6$ (r = 0.826**). Likewise, the relationship at the tillering stage was Y = $4950X^{-1} - 113.5$ (r = 0.965**).

There were no growth stage-dependent differences in adhesional force at a surface tension of 35 dyn/cm, although growth stage-dependent differences in contact angle were observed at this surface tension (Figure 7).

Considering the growth-stage-dependent differences in contact angle, the use of intact rice leaves at the tillering stage, rather than at the heading stage, would appear to be better for evaluating the wettability of pesticide spray solutions.

Contact angles and adhesional forces had a significant logarithmic relationship with surface tension (Figures 10, 11). However, the correlation coefficients (r) between adhesional force and surface tension (r = 0.879** to 0.903**) (Figure 11) were higher than those between contact angle and surface tension (r = 0.792** to 0.818**) (Figure 10). These

500X 5000X

M101 (Glabrous, japonica)

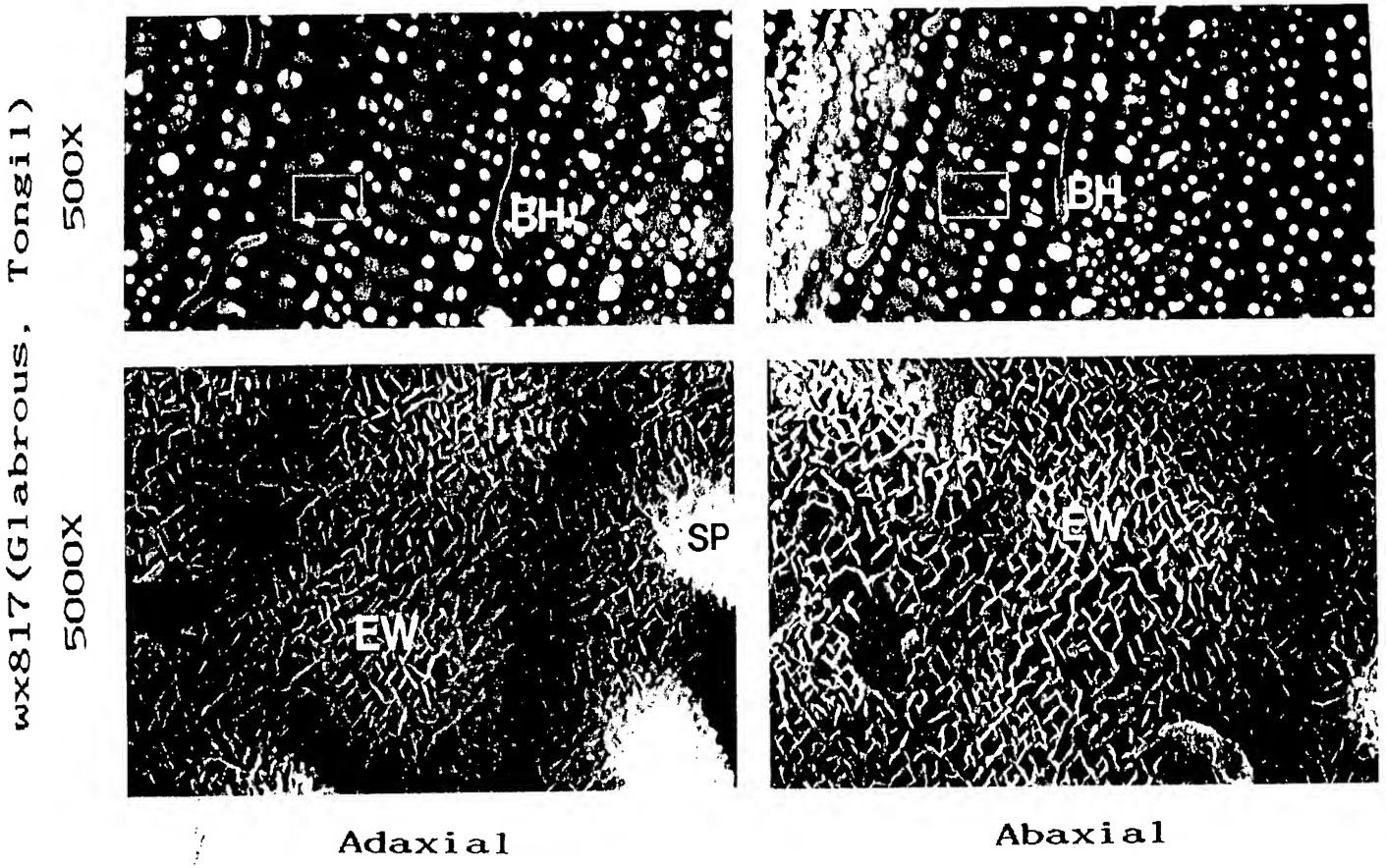

FIGURE 1. SEM photographs of glabrous rice leaf surfaces (flag leaf, varieties M101 and wx817). The lower part of the photographs represents a 10× magnification of the region marked by the white rectangular box. BH, bicellular microhair; EW, epicuticular wax; S, stomata; SP, small papillae.

5000X 500X

LK2-7 (Long-hairy, Japonica)

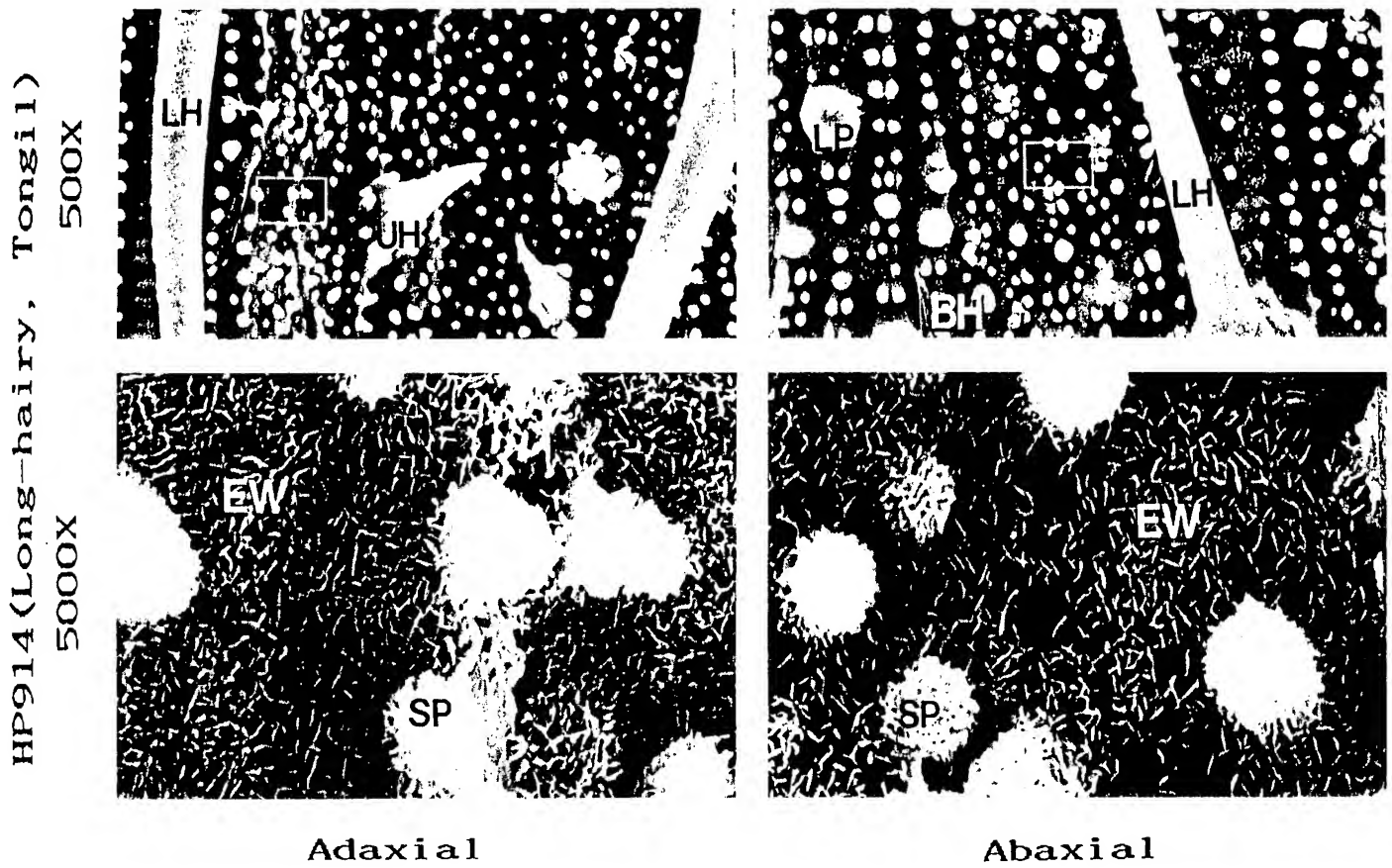

FIGURE 2. SEM photographs of long hairy rice leaf surfaces (flag leaf, varieties LK2-7 and HP914). The lower part of the photographs represents a 10x magnification of the region marked by the white rectangular box. BH, bicellular microhair; LH, long hair; LP, large inflated papillae; SP, small papillae; EW, epicuticular wax; UH, unicellular microhair.

ChuCheong (Pubescent, Japonica)

500X 5000X

FIGURE 3. SEM photographs of pubescent rice leaf surfaces (flag leaf, varieties Chucheong and Cheongcheong). The lower part of the photographs represents a 10× magnification of the region marked by the white rectangular box. BH, bicellular microhair; EW, epicuticular wax; LP, large inflated papillae; SP, small papillae; T, trichome; UH, unicellular microhair.

500X 5000X

HP857 (Water-wettable, Japonica)

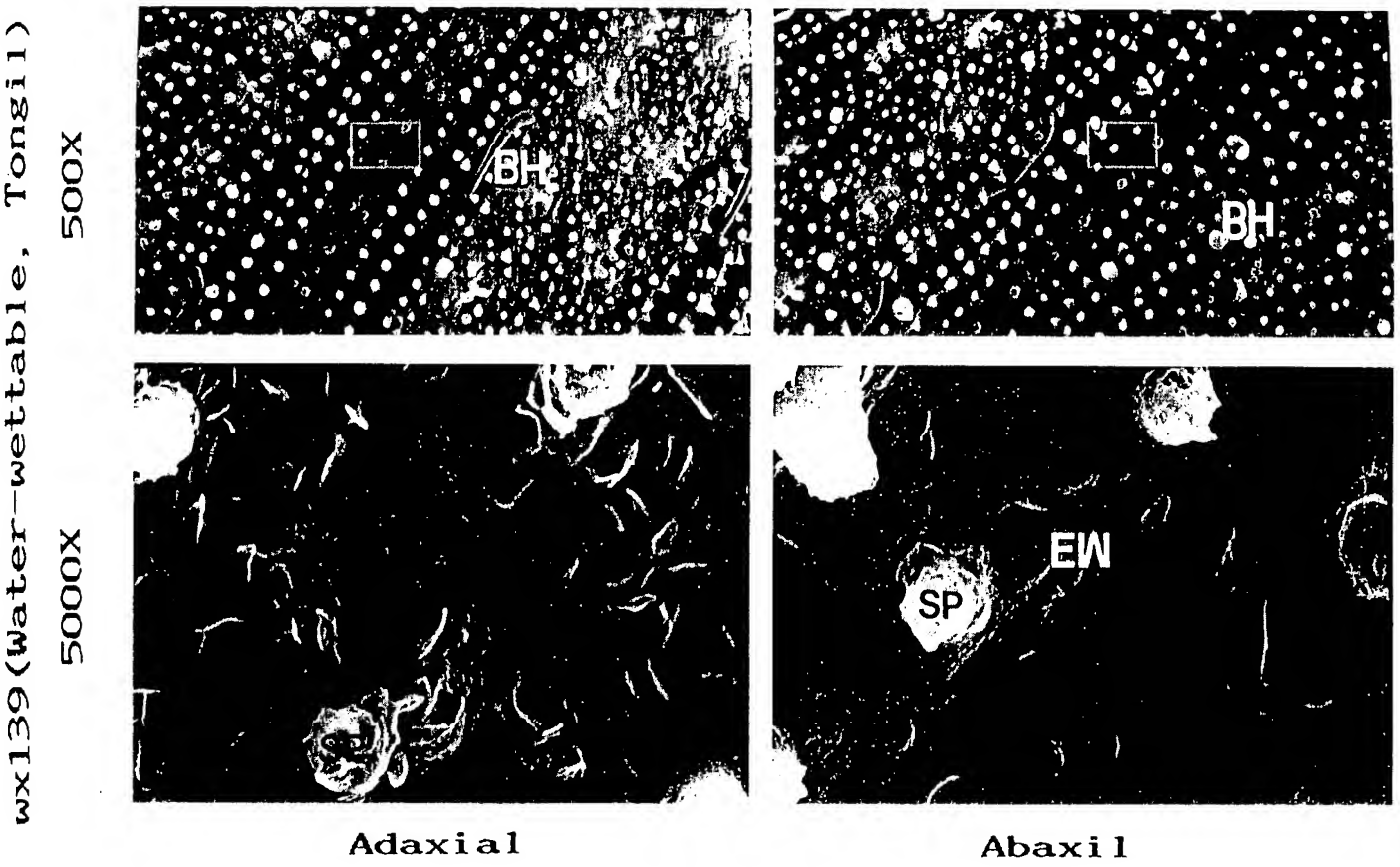

FIGURE 4. SEM photographs of water-wettable rice leaf surfaces (flag leaf, varieties HP857 and wx139). The lower part of the photographs represents a 10× magnification of the region marked by the white rectangular box. BH, bicellular microhair; EW, epicuticular wax; LP, large inflated papillae; SP, small papillae.

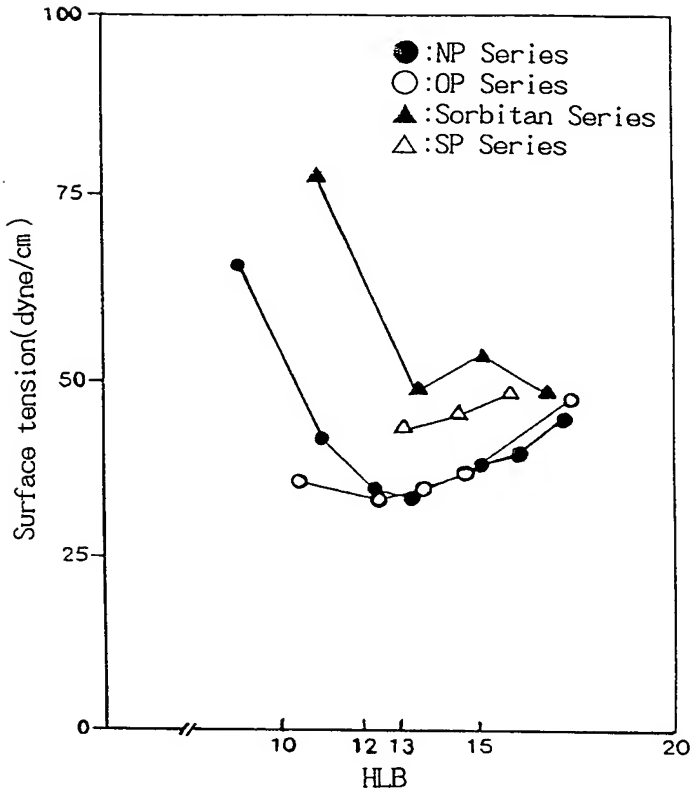

FIGURE 5. Relationship between surface tensions and HLB values among the nonionic surfactants used herein.

results suggest that adhesional force is a better criterion than contact angle for evaluating the wettability of nonionic surfactant solutions.

The contact angle increased more on the water-wettable than on the glabrous, long-hairy, and pubescent leaf surfaces, but the adhesional forces decreased less compared to the increase in the contact angle on the varieties of leaves (Figures 10 and 11). These results suggest that the wettability of intact leaf surfaces varies with the degree of wax coverage of the rice varieties rather than with differences in the fine morphology of the leaf surface (Figures 1 to 4).

Adhesional force had a significant relationship ($r = 0.948^{**}$) with contact angle for the eight rice varieties tested (Figure 12). The results shown in Figures 10 and 11 indicate strongly that adhesional force is a better criterion than contact angle in evaluating the wettability of nonionic surfactant solutions.

In conclusion, for increased wettability of the rice leaf surface, the HLB values of useful surfactants should lie in the range of 12 to 13, the surface tension should be about 35 dyn/cm, and a surfactant belonging to the NP or OP group is recommended. As a single criterion for selecting a proper surfactant, adhesional force appears to be the best choice.

** Highly significant statistically for the correlation r-value.

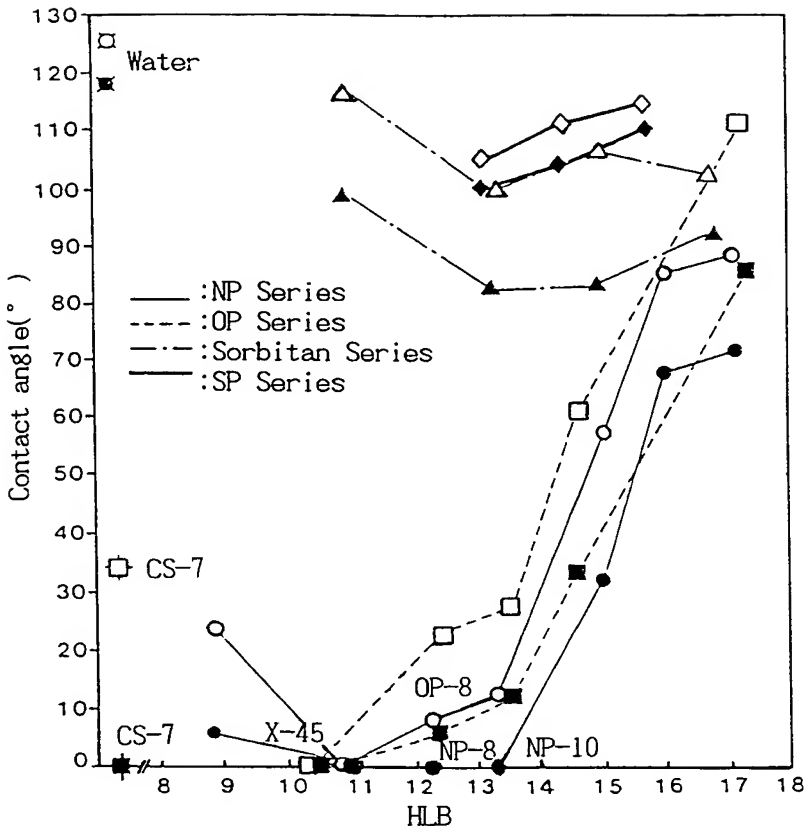

FIGURE 6. Relationship between HLB values of the nonionic surfactants and contact angles of 2-μl surfactant droplets measured on intact leaf surfaces of eight rice varieties at two different stages.

● NP series, heading
■ OP series, heading
▲ Sorbitan series, heading
◆ SP series, heading
✱ Distilled water, heading
✱ Triton CS-7, heading
○ NP series, tillering
□ OP series, tillering
△ Sorbitan series, tillering
◇ SP series, tillering
□ Distilled water, tillering
□ Triton CS-7, tillering

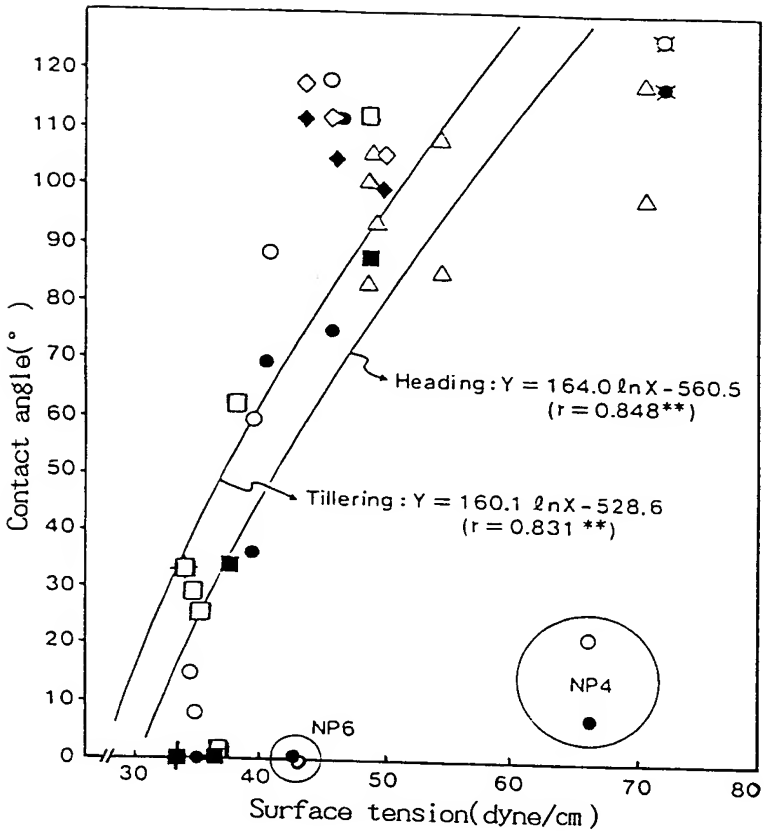

FIGURE 7. Relationship between surface tensions of 0.1% (w/v) nonionic surfactant solutions and contact angles measured on intact leaf surfaces of eight varieties at tillering and heading stages. NP-4 and NP-6 (denoted by a circle) have exceptionally low contact angles even though they have high surface tensions.

● NP series, heading
■ OP series, heading
▲ Sorbitan series, heading
◆ SP series, heading
✖ Distilled water, heading
✚ Triton CS-7, heading
○ NP series, tillering
□ OP series, tillering
△ Sorbitan series, tillering
◇ SP series, tillering
⌑ Distilled water, tillering
⌑ Triton CS-7, tillering

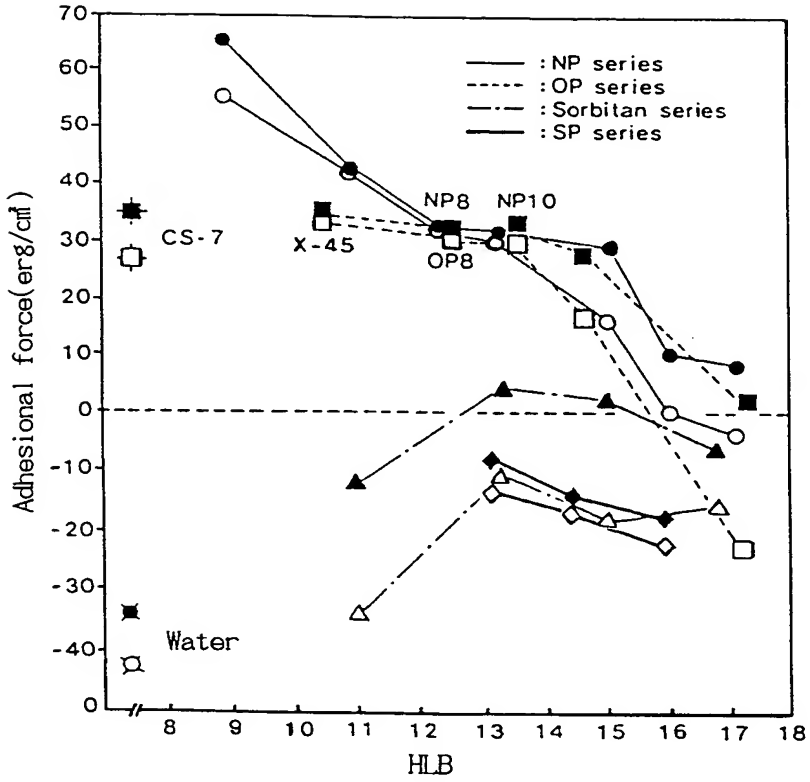

FIGURE 8. Relationship between HLB values of nonionic surfactants and adhesional forces of their 0.1% (w/v) water solutions on intact leaf surfaces of eight varieties at tillering and heading stages. Data are the average of adhesional forces ($Wa = rl \times \cos\theta$) calculated by measured contact angles (θ) and surface tensions (rl) on intact leaf surfaces of eight rice varieties.

● NP series, heading	○ NP series, tillering
■ OP series, heading	□ OP series, tillering
▲ Sorbitan series, heading	△ Sorbitan series, tillering
◆ SP series, heading	◇ SP series, tillering
Distilled water, heading	Distilled water, tillering
Triton CS-7, heading	Triton CS-7, tillering

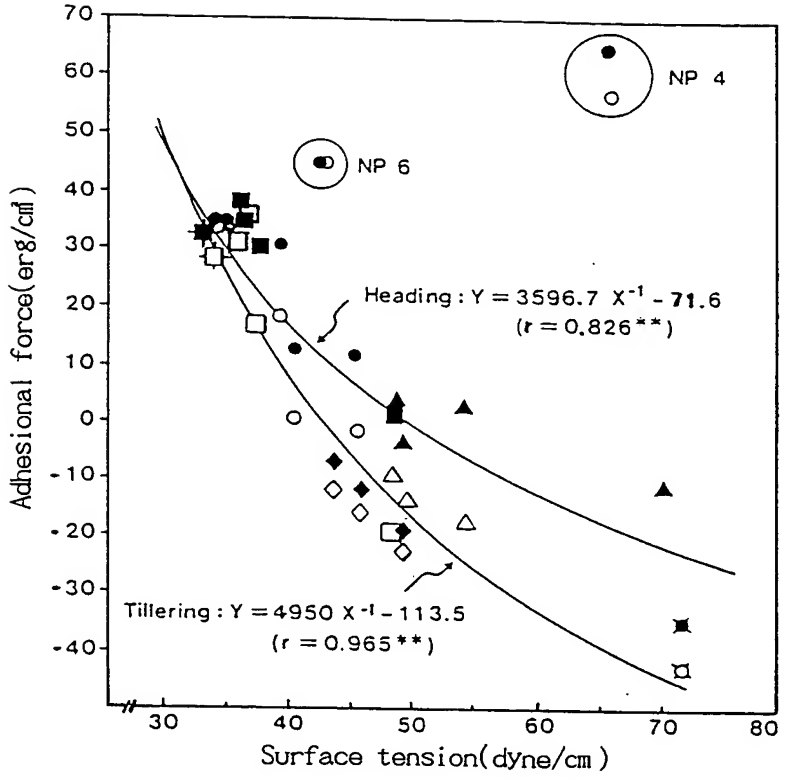

FIGURE 9. Relationship between surface tensions of 0.1% (w/v) nonionic surfactant solutions and the average adhesional forces calculated by their surface tension and cosine values of contact angles on intact leaf surfaces of eight varieties at tillering and heading stages. NP-4 and NP-6 (denoted by a circle) have exceptionally high adhesional forces even though they have high surface tensions.

- ● NP series, heading
- ■ OP series, heading
- ▲ Sorbitan series, heading
- ◆ SP series, heading
- ✖ Distilled water, heading
- ✸ Triton CS-7, heading
- ○ NP series, tillering
- □ OP series, tillering
- △ Sorbitan series, tillering
- ◇ SP series, tillering
- ⌑ Distilled water, tillering
- ⌬ Triton CS-7, tillering

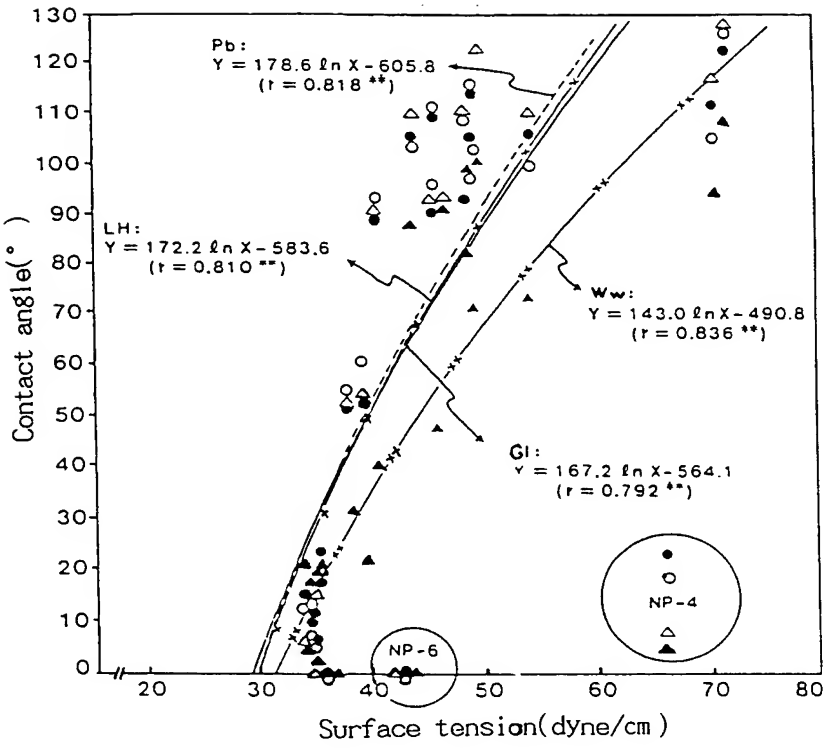

FIGURE 10. Relationship between surface tensions of 0.1% (w/v) nonionic surfactant solutions and contact angles on rice leaf surfaces among rice varieties classified by leaf surface property. Data are the averages of 2-μl surfactant droplets measured on intact leaf surfaces. NP-4 and NP-6 (denoted by a circle) have exceptionally low contact angles even though they have high surface tensions.

● Glabrous (Gl) ○ Long-hairy (LH)
▲ Pubescent (Pb) △ Water wettable (Ww)

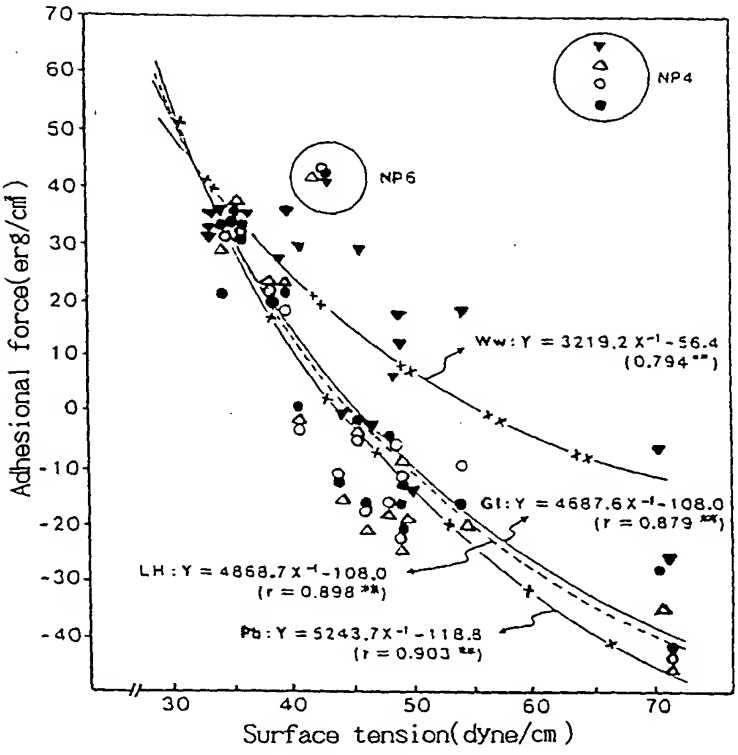

FIGURE 11. Relationship between surface tensions of 0.1% (w/v) nonionic surfactant solutions and adhesional forces on rice leaf surfaces among rice varieties classified by leaf surface property. Data are the averages of adhesional forces calculated by surface tensions of surfactant solutions and their contact angles on intact leaf surfaces. NP-4 and NP-6 (denoted by circle) have exceptionally high adhesional forces even though they have high surface tensions.

○ Glabrous (Gl) △ Pubscent (Pb)
● Long-hairy (LH) ▼ Water wettable (Ww)

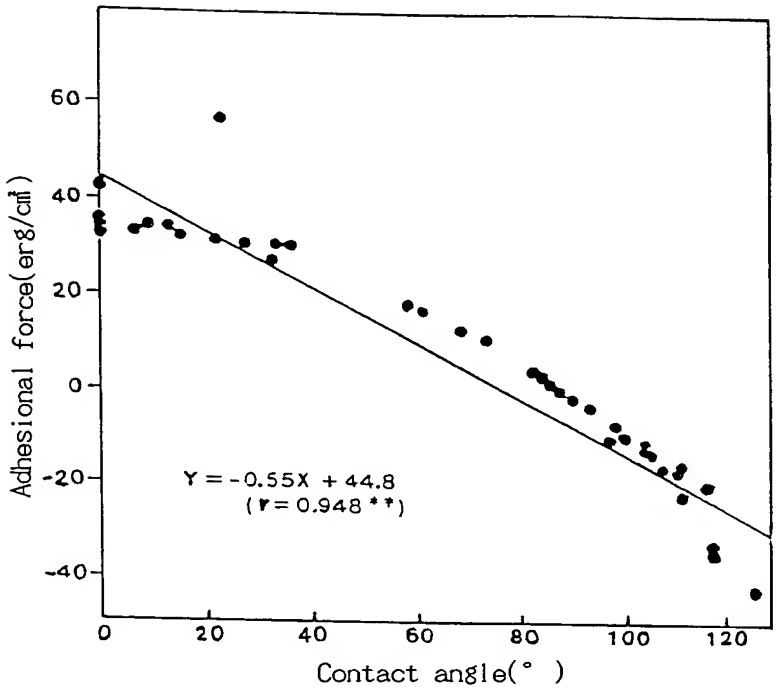

FIGURE 12. Relationship between contact angles on intact leaf surfaces and adhesional forces calculated by surface tensions of 0.1% (w/v) nonionic surfactant solutions and their contact angles on intact rice leaf surfaces. Data are the averages for the eight rice varieties.

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Chapter 4

SURFACTANT-INDUCED ETHYLENE EVOLUTION AND PIGMENT EFFLUX FROM BEET (*BETA VULGARIS* L.) ROOT TISSUE

Hiroyuki Matsui, Warren E. Shafer, and Martin J. Bukovac

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ABSTRACT

The physiological activity of selected surfactants commonly used in agriculture and/or representing selected chemistries was determined using beet root tissue (*Beta vulgaris* L.) as a model system. Biological activity was assessed by measuring simultaneously the effect of the surfactant on the induction of ethylene evolution and on membrane integrity. Tissue discs were incubated in appropriate test solutions at pH 7 for 3 h at 30°C in the dark. Ethylene was determined by gas chromatographic analysis of headspace samples, and membrane integrity was assessed by spectrophotometrically measuring betacyanin efflux into the incubation medium. Surfactants either promoted, had no effect on, or suppressed ethylene evolution, while they either increased or had no effect on membrane permeability. Responses of both systems were related to surfactant chemistry and concentration. Of 29 surfactants evaluated, 18 enhanced both ethylene evolution and betacyanin efflux, while only 5 enhanced ethylene production without affecting membrane integrity. Five enhanced betacyanin efflux but depressed ethylene evolution. One had no significant effect on either parameter. In general, polyoxyethylene derivatives of octylphenol, nonylphenol, and linear alcohols (C_{9-11}, C_{12-15} hydrophobe) decreased in biological activity with increasing oxyethylene chain length. For most surfactants, the minimum effective concentrations for induction of ethylene evolution and pigment efflux were 0.01 and 0.05%, respectively. The relationships between surfactant chemistry and ethylene evolution and membrane integrity are discussed.

I. INTRODUCTION

Surfactants are commonly used in agrichemical formulations to stabilize emulsions and/or suspensions,[4] increase retention and spreading of spray droplets,[12,13] and enhance penetration of the active ingredient.[12] Unfortunately, many surfactants possess biological activity in plant systems,[34] including inhibition of root,[15,17,35] coleoptile,[47] callus,[10] suspension cultures,[7] and frond growth/development,[6] inhibition of seed germination,[18] and the induction of cellular necrosis and subsequent tissue collapse in leaf organs.[21-23] While several mechanisms of surfactant action in plant tissues have been identified, the most common appears to be disruption of membrane integrity.[3,8,11,16,30,44] In fact, surfactants are frequently used to solubilize the lipid:protein components of membranes.[16] In light of this, considerable effort has focused on quantifying the impact of surfactants on membrane integrity. The most commonly used method to quantify the disruption of membrane integrity has been to measure pigment and/or ion efflux.[5,29,32,40,41,45] Surfactant-enhanced ethylene production is also a membrane-related phenomenon.

Lownds and Bukovac[23] and Stevens and Bukovac[43] studied the induction of ethylene evolution in leaf tissue by surfactants and reported that ethylene evolution was inversely related to polyoxyethylene (POE) chain length for a series of nonionic (octylphenol derivatives) surfactants. Numerous investigators[5,29,32,45] have utilized betacyanin efflux from beet root tissue to assess the toxicity of selected nonionic, anionic, and cationic surfactants. In general, toxicity was related to surfactant chemistry (ionic surfactants were typically more toxic than nonionic surfactants), concentration (toxicity increased with increasing concentration), and in the case of POE surfactants, POE content (toxicity was inversely related to POE chain length).

Based on the data presented in the preceding text, it is clear that the need to incorporate a surfactant(s) in an agrichemical formulation/spray solution should be balanced against the possible deleterious biological activity of the surfactant(s) in plant systems. To realistically achieve this goal, a basic understanding of surfactant chemistry/biological activity relationships must be obtained. In addition, a relatively simple bioassay system should be available

so that a candidate surfactant can be rapidly tested for possible adverse responses in plant tissues.

The beet root disc assay offers two important advantages over the leaf/ethylene assays mentioned above, namely, rapidity (a few hours to complete vs. a few days) and the absence of a cuticular barrier to uptake/penetration. With respect to the latter point, although there is mounting evidence that surfactants penetrate foliage/plant cuticles,[36,42,43] it is important to distinguish between differences in penetration and differences in innate biological activity.

Given the profound effects of surfactants on both ethylene production and pigment efflux in plant tissues, as well as the advantages of the beet root disc assay, we were interested in determining whether both responses could be studied simultaneously using beet root tissue. Once the assay system was optimized, we evaluated a number of commercially important surfactants, varying in chemistry, to probe surfactant chemistry/biological activity relationships. The results of our studies are reported herein.

II. MATERIALS AND METHODS

A. PLANT MATERIAL/TISSUE PREPARATION

Fresh beets (*Beta vulgaris* L.) were purchased locally. After removing the apical and basal portions (each about 25% of the total root), 6-mm diameter cylinders were removed longitudinally from between the vascular rings of the median segment using a cork borer. The excised cylinder was then sectioned transversely into 2-mm-thick discs using a hand microtome. Only discs free of visual defects or distortion were used.

B. ASSAY PROCEDURE

Twenty discs were placed on filter paper in 25-ml Erlenmeyer flasks containing 1 ml of treatment solution. The 1-ml solution volume was selected because adequate tissue exposure was obtained without the risk of the discs floating/bumping together or being submerged. Six replicate flasks were used per treatment. The flasks were immediately sealed with rubber septa and incubated in the dark. The specific assay conditions are described below (see Section II.C).

Ethylene was measured in a 1-ml headspace gas sample by gas chromatography.[23] Betacyanin efflux into the treatment (incubation) solution was quantified by removing the discs from each flask, adding 9 ml of 1% HCl:methanol, and then reading the absorbance at 540 nm with a spectrophotometer (Bausch & Lomb, Model 20).

Due to root to root variability in betacyanin content, absolute absorbance data from a set of discs could not be used to quantify surfactant effects. Instead, 120 representative discs were selected from the total sample pool immediately prior to initiating each experiment and divided into six replicates of 20 discs each. Each replicate was exhaustively extracted with 50 ml of 1% HCl:methanol and the absorbance (540 nm) measured. The percentage of betacyanin efflux induced by the respective treatment was then calculated according to:

$$\% \text{ betacyanin efflux} = \frac{\text{amount betacyanin in incubation medium}}{\text{amount betacyanin extracted from discs}} \times 100 \qquad (1)$$

C. ASSAY DEVELOPMENT/OPTIMIZATION

Ethylene production and pigment efflux were optimized to ensure that treatment responses could be quantified. Several experimental parameters of critical importance to our assay were examined. These included ethylene substrate (1-aminocyclopropane-1-carboxylic acid, ACC; Calbiochem) concentration, disc-rinsing time, buffer pH, incubation temperature, incubation time-course, and buffer composition. Given the large number of variables and

our interest in sequentially investigating each individually, it was necessary to arbitrarily set certain test conditions until the optimal condition was established in the appropriate range-finding study.

1. Effect of Ortho X-77 Concentration

Due to the marked effect surfactants have on solution properties and the possibility that such changes might influence the results obtained during our method-development experiments, Ortho X-77 (Table 1) was selected as the standard surfactant for optimization of our assay. Three concentrations (0.01, 0.1, and 1.0% w/v) were examined to establish the effect of concentration on ethylene evolution and pigment efflux. The assay conditions were: disc rinse period, 15 h; 100 m*M* phosphate buffer, pH 6.7; temperature, 25°C; incubation time, 3 h. Ethylene evolution increased approximately 2.5-fold between controls (no Ortho X-77) and the 1.0% treatment (data not shown). There was no pigment efflux from controls or the 0.01% Ortho X-77 treatment. However, pigment efflux increased from 4 to 13% as the Ortho X-77 concentration was increased from 0.1 to 1.0%. Based on these data, we selected 1.0% Ortho X-77 as a standard concentration to establish the remaining parameters.

2. Effect of Disc-Rinsing Time

Rinsing the root discs with distilled water (20°C) was necessary to remove pigment remaining on the cut surfaces which could interfere with data interpretation. Sets of discs were rinsed for selected periods of time (3 to 15 h, at 3-h intervals) and then incubated. The assay conditions were: 100 m*M* phosphate buffer, pH 6.7; temperature, 25°C; incubation time, 3 h. Ethylene production increased linearly as rinse times were increased to 9 h, but then decreased slightly as rinse times were increased to 15 h (data not shown). Pigment efflux decreased slightly with an increase in rinse times. Based on these data and scheduling convenience, we selected 10 to 11 h as our routine disc rinse period.

3. Effect of ACC Concentration

ACC is the primary substrate for ethylene biosynthesis in plant tissue.[48,49] Due to concerns about the depletion of endogenous ACC during disc rinsing, we evaluated the need to supply ACC (10 μ*M* to 100 m*M*) in the treatment solution. The assay conditions were: disc rinse period, 11 h; 100 m*M* phosphate buffer, pH 7.0; temperature, 25°C; incubation time, 3 h. Ethylene evolution increased with increasing ACC concentration, reaching a maximum at 10 m*M* (Figure 1A). ACC did not induce pigment efflux. When combined with 1.0% Ortho X-77, ethylene production was greater for all ACC concentrations examined, with maximal production still at 10 m*M*. ACC had no significant effect on the amount of pigment efflux induced by Ortho X-77. Based on these data, 10 m*M* ACC was routinely included in the remaining assays.

4. Effect of Buffer pH

The effect of pH was examined over the range of 3 to 9 using 100 m*M* phosphate buffer. The assay conditions were: disc rinse period, 11 h; Ortho X-77 concentration, 1.0%; ACC concentration, 10 m*M*; temperature 25°C; incubation time, 3 h. Ethylene production increased linearly from pH 3 to 7 and then declined at pH 8 and 9 (Figure 1B). Pigment efflux followed a similar pattern. Thus, buffer pH was routinely set at 7.0.

5. Effect of Temperature

Temperature effects, during incubation, on ethylene production and pigment efflux were tested over the range of 20 to 40°C. The assay conditions were: disc rinse period, 10 h; 100 m*M* phosphate buffer, pH 7.0; Ortho X-77 concentration, 1.0%; ACC concentration, 10

TABLE 1
Selected Chemical Characteristics of Surfactants Examined[a]

Trade name	Code #	Source[b]	POE[c,d]	MW[d]	HLB	cmc (%)
Representative Nonionic Surfactants						
Ortho X-77	1	3	NA[e]	NA	NA	0.01
Triton X-100	2	6	9.5	628	13.5	0.019
Triton X-405	3	6	40.0	1966	17.9	0.17
Tween 20	4	5	20.0	1244	16.7	0.006
Tween 80	5	5	20.0	1370[f]	15.0	0.004[g]
Representative Anionic Surfactants						
Duponol	6	4	NA	288	NA	0.24
Aerosol OT	7	1	NA	444	NA	0.03
Aerosol OT-B	8	1	NA	444	NA	0.03[h]
Representative Cationic Surfactants						
Arquad 2C-75	9	2	NA	447	NA	0.01
Arquad C-50	10	2	NA	278	NA	0.009
Nonionic Surfactant Series						
Neodol 91 Series		7				
91-6	11		6.0	425	12.4	0.025
91-8	12		8.0	529	14.0	0.027
91-10	13		10.0	600	14.7	0.029
91-12	14		12.0	680	15.3	0.031
91-20	15		20.0	1040	16.9	0.039
Neodol 25 Series		7				
25-3	16		3.0	336	7.9	0.0001
25-7	17		7.0	522	12.2	0.0009
25-9	18		9.0	610	13.3	0.0018
25-12	19		12.0	729	14.4	0.0027
25-30	20		30.0	1548	17.1	0.016
Triton X Series		6				
X-15	21		1.0	250	3.6	0.001
X-35	22		3.0	338	7.8	0.004
X-45	23		5.0	426	10.4	0.005
X-114	24		7.5	536	12.4	0.009
X-102	25		12.5	756	14.6	0.029
X-305	26		30.0	1526	17.3	0.11
Triton N Series		6				
N-42	27		4.0	405	9.1	0.0021[g]
N-57	28		5.0	440	10.0	0.0025
N-150	29		15.0	880	15.0	0.0083

[a] Data from References 21, 31, and 43.

[b] Source codes: 1 = American Cyanamid, Wayne, NJ; 2 = Armac Co., Chicago, IL; 3 = Chevron Chemical Co., Richmond, CA; 4 = E. I. du Pont de Nemours & Co., Wilmington, DE; 5 = ICI Americas, Inc., Wilmington, DE; 6 = Rohm & Haas, Philadelphia, PA; 7 = Shell Oil Co., Houston, TX.

[c] Polyoxyethylene content.

[d] Average values.

[e] Either not applicable or not available.

[f] Personal communication, M. Patel (ICI Americas, Inc., Wilmington, DE).

[g] Unpublished data, A. Heredia (Michigan State University, East Lansing).

[h] The assumption was made that the presence of sodium benzoate in the Aerosol OT-B (the only difference between OT and OT-B) did not significantly affect the cmc.

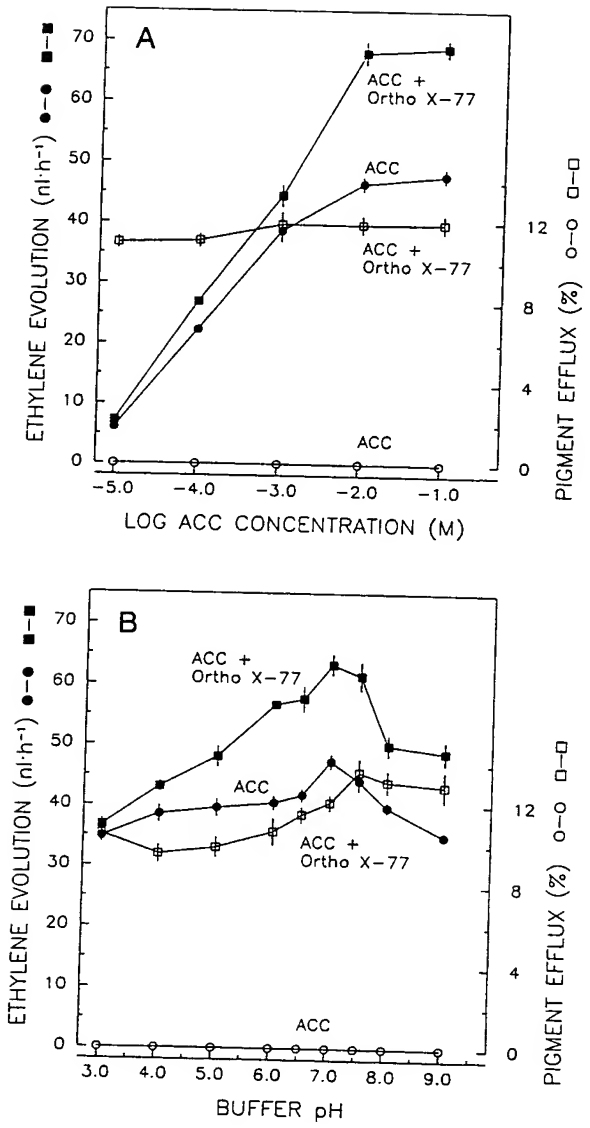


FIGURE 1. Effect of ACC concentration (A) and pH of incubation medium (B) on ethylene evolution and pigment efflux from beet root tissue. See text for specific assay conditions.

m*M*; incubation time, 3 h. Ethylene production resembled a bell-shaped curve, with a maximum at 30°C (Figure 2). Pigment efflux increased slightly between 20 and 30°C, but increased dramatically at 35 and 40°C. Therefore, 30°C was routinely adopted for all assays.

6. Time Course of Ethylene Production and Pigment Efflux

Ethylene evolution and pigment efflux were monitored over a 24-h period to document time-dependent responses. Assay conditions were: disc rinse period, 10 h; 100 m*M* phosphate buffer, pH 7.0; Ortho X-77 concentration, 1.0%; ACC concentration, 10 m*M*; temperature,

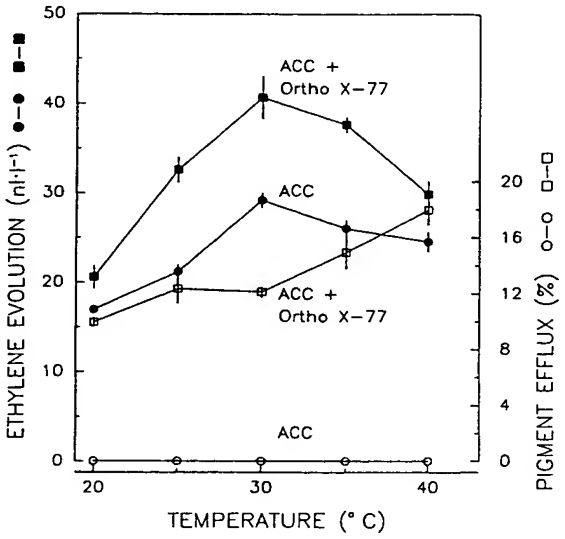


FIGURE 2. Effect of assay temperature on ethylene evolution and pigment efflux from beet root tissue. See text for specific assay conditions.

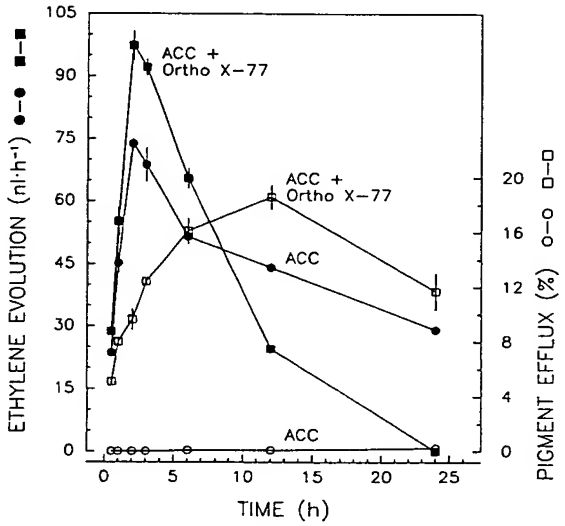


FIGURE 3. Time course of ethylene evolution and pigment efflux from beet root tissue. See text for specific assay conditions.

30°C; incubation time, 3 h. Initially, ethylene levels increased sharply, reaching a maximum at 2 h (Figure 3). Thereafter, ethylene production decreased steadily and approached background levels after 24 h. Pigment efflux also increased sharply at first, reaching a maximum at 12 h, and then decreased after 24 h. Based on these data and scheduling convenience, we selected 3 h as our standard assay period. While this time interval was not optimum for pigment efflux, sufficient efflux of pigment occurred by 3 h for quantification.

TABLE 2
Effect of Hydrophobe for Two Alkyl (12EO) and Two Aryl (5EO) Surfactants on Ethylene Evolution (% of Control)

Hydrophobe	Concentration (%)					
	0.01	0.05	0.1	0.5	1.0	2.0
C_{9-11} linear alcohol[a] (Neodol 91-12)	99 (5)[b]	158 (3)	144 (5)	118 (3)	106 (4)	ND[c]
C_{12-15} linear alcohol[a] (Neodol 25-12)	119 (4)	ND	133 (5)	149 (3)	145 (6)	125 (4)
Octylphenol (Triton X-45)	127 (5)	140 (8)	130 (4)	108 (8)	104 (4)	ND
Nonylphenol (Triton N-57)	148 (4)	155 (4)	157 (6)	145 (4)	138 (7)	ND

[a] Mixture of hydrophobe chain lengths (C_{9-11}, C_{12-15}).
[b] Mean (SE).
[c] Not determined.

7. Buffer Composition

Four buffers (Tris, borate, citrate, and phosphate) were tested at 100 m*M* and pH 7.0. The assay conditions were: disc rinse period, 10 h; Ortho X-77 concentration, 1.0%; ACC concentration, 10 m*M*; temperature, 30°C; incubation time, 3 h. Both Tris and borate buffers yielded significantly greater amounts of ethylene than phosphate and citrate (data not shown). Pigment efflux induced in the Tris and borate buffer treatments was significantly lower than with the citrate and phosphate buffers. Since phosphate buffer represented an intermediate response for both parameters, it was selected for our assay.

8. Method Development/Optimization Summary

Based on these experiments, the following standard assay system was adopted for the surfactant studies: discs were rinsed (distilled water, 20°C) for 10 h prior to use and incubated (in the dark) at 30°C for 3 h with 10 m*M* ACC in 100 m*M* phosphate buffer at pH 7.0.

D. SURFACTANTS

The surfactants utilized herein were obtained from commercial sources (Table 1). Trade names and general chemistries (in parentheses) for the surfactants/surfactant series used herein are Ortho X-77 (mixture of alkyaryl glycols, free fatty acids, and isopropanol), Triton X series (polyoxyethylene derivatives of octylphenol), Triton N series (polyoxyethylene derivatives of nonylphenol), Tween 20 (polyoxyethylene [20] sorbitan monolaurate), Tween 80 (polyoxyethylene [20] sorbitan monooleate), Duponol (sodium lauryl sulfate), Aerosol OT/Aerosol OT-B (dioctyl sodium sulfosuccinate; Aerosol OT-B is the same as Aerosol OT except that it contains sodium benzoate), Arquad 2C-75 (dicoco dimethyl ammonium chloride), Arquad C-50 (monococo trimethyl ammonium chloride), Neodol 91 series (polyethoxylated C_{9-11} linear primary alcohol derivatives), and Neodol 25 series (polyethoxylated C_{12-15} linear primary alcohol derivatives). All polyethoxylated surfactants (Table 1, POE column) were mixtures of ethoxymers, with the mole distribution following a Poisson distribution.[39,46] Also, for the Neodol linear alcohol series, the hydrophobe length ranged from C_{9-11} or C_{12-15}. No attempt was made to further purify the surfactants prior to use. Corrections were made for percent a.i. differences when necessary. All solution concentrations were based on weight/volume.

E. DATA PRESENTATION

The data in Tables 2 and 3 and Figures 1 through 6 are means of six replicates (20 discs/replicate) with their respective standard error (SE) values. In the figures where SE

TABLE 3
Effect of Hydrophobe for Two Alkyl (12EO) and Two Aryl (5EO) Surfactants on Pigment Efflux (%)

Hydrophobe	Concentration (%)					
	0.01	0.05	0.1	0.5	1.0	2.0
C_{9-11} linear alcohol[a] (Neodol 91-12)	0[b]	0	0.4 (<0.1)[b]	7.6 (0.5)	16.4 (0.7)	ND[c]
C_{12-15} linear alcohol[a] (Neodol 25-12)	0	ND	0.7 (0.1)	1.0 (<0.1)	3.3 (0.1)	7.2 (0.4)
Octylphenol (Triton X-45)	0.2 (<0.1)	3.3 (0.3)	8.7 (0.6)	11.9 (0.7)	16.3 (0.7)	ND
Nonylphenol (Triton N-57)	0.2 (<0.1)	1.6 (0.1)	2.8 (0.2)	5.9 (0.4)	8.5 (0.5)	ND

[a] Mixture of hydrophobe chain lengths (C_{9-11}, C_{12-15}).
[b] Mean (SE).
[c] Not determined.

values are not shown, they were smaller than the data symbol. Ethylene data are presented as either the amount of ethylene evolved (nl/h; Figures 1 to 3) or as a percentage of the average amount of ethylene produced by control (i.e., no surfactant) discs (Figures 4 to 6). Betacyanin efflux data are presented as percent values (see Equation 1).

III. RESULTS AND DISCUSSION

Two primary goals were established for these studies, namely: (1) optimize a bioassay system which would be useful for screening candidate surfactants for possible deleterious biological activity and (2) investigate surfactant chemistry/biological activity relationships for a selection of commonly used surfactants. While these goals have also been the focus of others,[6,7,35,41] including studies specifically utilizing beet root tissue,[5,29,32,45] our studies are unique because they stressed the effects of surfactants on two membrane-associated phenomena in the same plant system.

Before detailing our results, a brief overview of ethylene biosynthesis, as well as pigment localization in beet root cells, should be helpful. Ethylene synthesis in plant tissues is believed to be as follows:[19,49] methionine, the general precursor of ethylene, is converted into *S*-adenosylmethionine (SAM); SAM is then converted, via the enzyme ACC synthase, to ACC; and finally, ACC is converted, via the ethylene-forming enzyme (EFE), to ethylene.

Two comments regarding this sequence are in order. First, the conversion of SAM to ACC is the rate-limiting step.[48] In other words, ACC synthase activity controls ethylene biosynthesis. As mentioned in Section II, we supplied ACC to the beet root discs in our assay to ensure that substrate would not be limiting. Second, the EFE has not been isolated and/or characterized to date.[25] Based on several lines of evidence,[27,28] including studies on surfactant disruption of membranes,[1,2,24,33] the EFE is a highly organized, membrane-bound enzyme. The EFE is probably associated with both the plasma membrane and tonoplast,[49] although the cell wall may also be involved.[26] For a detailed discussion of ethylene biosynthesis, the reader is referred to References 20, 25, 48, and 49.

In our bioassay, we monitored the efflux of betacyanins, a group of reddish-violet pigments.[14] They are small molecular weight, nitrogen-containing compounds concentrated in the vacuole. Perhaps the most well-known betacyanin is betanin, which was first crystallized from beet root tissue.[37] In mature beet root cells, the vacuole comprises approximately

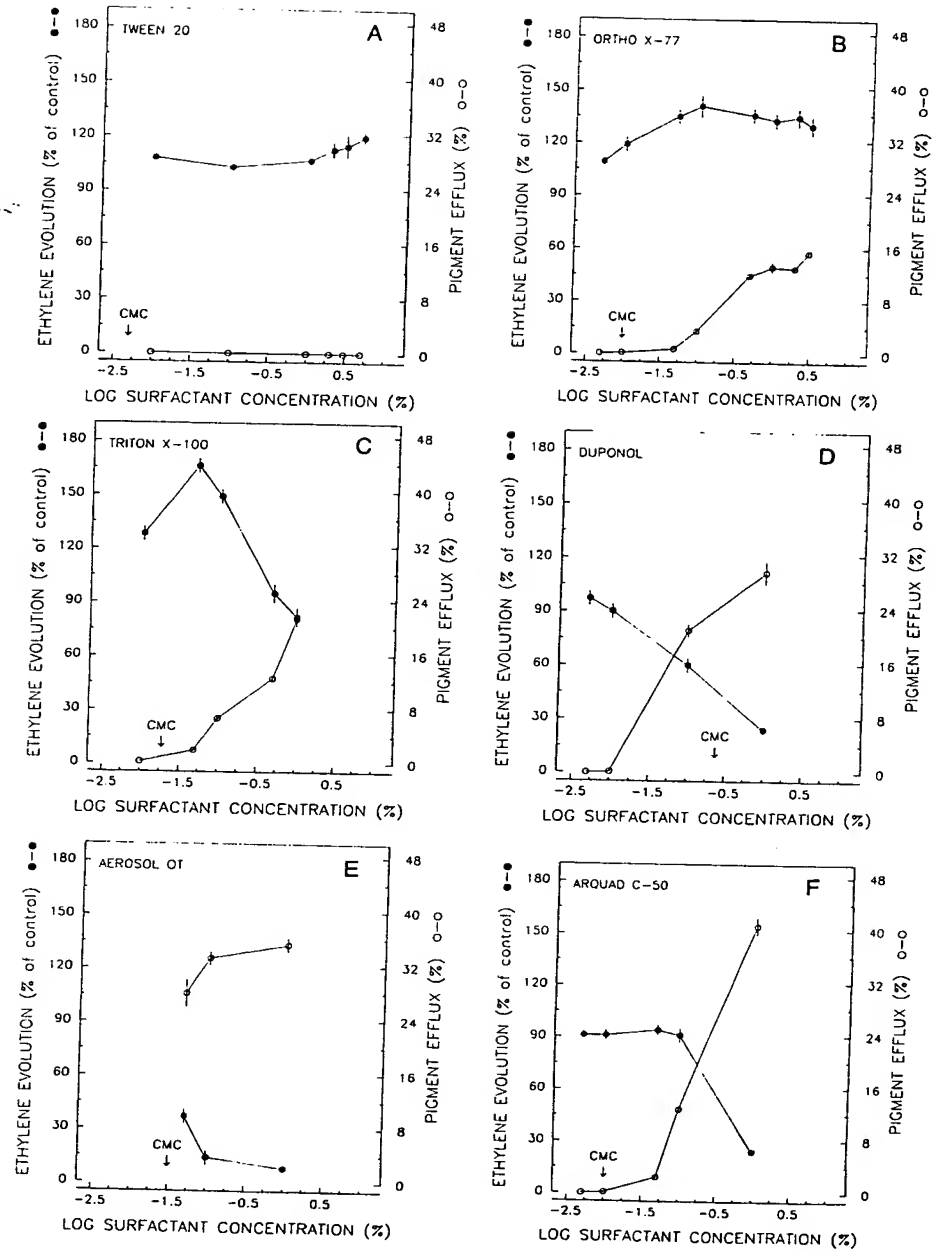


FIGURE 4. Effect of Tween 20 (A), Ortho X-77 (B), Triton X-100 (C), Duponol (D), Aerosol OT (E), and Arquad C-50 (F) concentration on ethylene evolution and pigment efflux from beet root tissue. Assay conditions: 25°C, pH 7.0, 10 m*M* ACC, 3-h incubation. Average control (no surfactant) ethylene evolution values (nl/h) for Tween 20, Ortho X-77, Triton X-100, Duponol, Aerosol OT, and Arquad C-50 were (SE values in parentheses) 40.0 (1.2), 33.7 (1.3), 33.9 (1.5), 34.3 (1.6), 28.7 (1.0), and 34.3 (1.1), respectively.

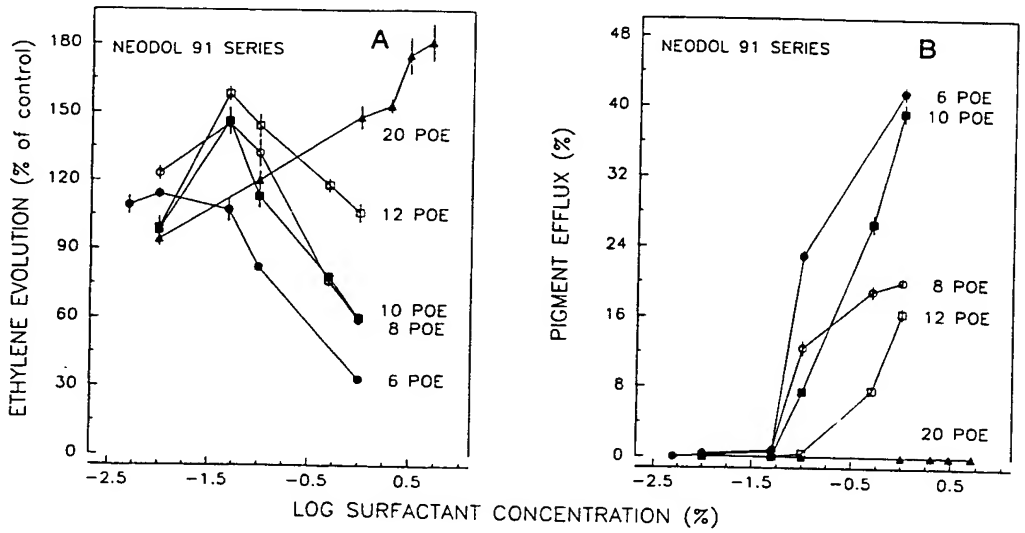


FIGURE 5. Effect of polyoxyethylene chain length for the Neodol 91 surfactant series, as a function of concentration, on ethylene evolution (A) and pigment efflux (B) from beet root tissue. Assay conditions: 25°C, pH 7.0, 10 m*M* ACC, 3-h incubation. Average control (no surfactant) ethylene evolution value (nl/h) for Neodols 91-6 and 91-8 was (SE value in parentheses) 40.0 (1.2); for Neodols 91-10, 91-12, and 91-20, the value was 37.3 (1.4).

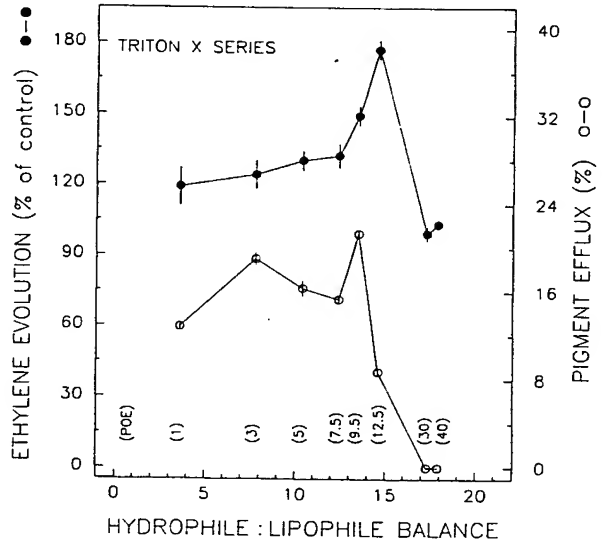


FIGURE 6. Relationship between hydrophile:lipophile balance (HLB) and ethylene evolution and pigment efflux from beet root tissue. Surfactant concentration: 0.1% for ethylene data, 1.0% for pigment data. Assay conditions: 25°C, pH 7.0, 10 m*M* ACC, 3-h incubation. Average control (no surfactant) ethylene evolution values (nl/h) for Tritons X-15, X-35, X-45, X-114, X-100, X-102, X-305, and X-405 were (SE value in parentheses) 32.3 (1.3), 32.3 (1.3), 28.7 (1.0), 30.4 (1.4), 33.9 (1.5), 30.4 (1.4), 28.7 (1.0), and 30.2 (0.6), respectively.

70% of the cellular volume (as cited in Reference 29). Although some betacyanin may be found in the cytoplasm because it is the probable site of biosynthesis, the tonoplast is considered to be the main barrier to betacyanin efflux (as cited in Reference 29).

Thus, both responses studied herein are intimately dependent on membrane integrity. If membrane disruption occurs, one would anticipate a loss of EFE activity (and subsequently a decrease in ethylene production) and a leakage of cytoplasmic/vacuolar compounds (pigment efflux) into the incubation medium.

The effect of surfactant concentration on ethylene evolution (percent of control) and pigment efflux (percent) data for six representative surfactants are presented in Figure 4 (A through F). These surfactants represent three different classes of chemistry (based on net molecular charge). Tween 20, Ortho X-77, and Triton X-100 (Figures 4A through C) are nonionic, Duponol and Aerosol OT (Figures 4D and E) are anionic, and Arquad C-50 (Figure 4F) is cationic. These surfactants were selected for presentation because they illustrate the five main response patterns observed in our studies.

Each of the three nonionic surfactants demonstrated unique concentration-dependent behavior. Tween 20 did not induce pigment efflux at any of the concentrations examined (Figure 4A). Ethylene evolution increased slightly over control values, especially at concentrations in excess of 1.0%. With Ortho X-77 at a concentration below the critical micelle concentration (cmc), there was essentially no effect on ethylene evolution or pigment efflux (Figure 4B). As the Ortho X-77 concentration was increased above the cmc, both ethylene evolution and pigment efflux increased. Ethylene evolution reached a plateau as the concentration approached 0.1%, whereas pigment efflux continued to increase with an increase in concentration. With Triton X-100, the ethylene evolution curve was biphasic (Figure 4C). There was a slight increase in ethylene evolution at a pre-cmc concentration of Triton X-100 without an accompanying effect on pigment efflux. While pigment efflux steadily increased with increasing Triton X-100 concentration above the cmc, ethylene evolution reached a peak at 0.05% and then decreased dramatically.

The remaining two response patterns are illustrated by an anionic and cationic surfactant, respectively. Duponol inhibited ethylene evolution but induced marked pigment efflux at concentrations well below the cmc (Figure 4D; although data were limited, Aerosol OT [Figure 4E] also demonstrated this type of response). For Arquad C-50, ethylene evolution was slightly lower than controls at the lowest concentration tested (0.005%) and held constant until the surfactant concentration exceeded 0.1%, then decreased dramatically (Figure 4F). Pigment efflux did not occur until the cmc was exceeded, but then increased linearly with increasing concentration.

All 29 surfactants studied can be categorized into one of the five qualitative response patterns described above (Figure 4 representative in parentheses): (Tween 20), Tritons X-305 and X-405, Neodols 25-30 and 91-20, and Tween 80; (Ortho X-77), Neodol 25-3; (Triton X-100), Tritons X-15, X-35, X-114, and X-102, Tritons N-42, N-57, and N-150, Neodols 91-6, 91-8, 91-10, 91-12, 25-7, 25-9, and 25-12; (Duponol/Aerosol OT), Aerosol OT-B; (Arquad C-50), Arquad 2C-75. Given space limitations, it is not possible to present all of our data. Therefore, for some surfactants, this listing of qualitative responses is all that is given. It should be noted that although we attempted to thoroughly test all of the surfactants used in this study, it is possible that we missed an enhancement and/or inhibition response because either our concentration range was too narrow or our concentration increments were too large.

The Neodol 91 series of linear alcohol (mixture of C_{9-11} hydrophobe chain lengths) surfactants was selected to illustrate the effect of POE content, as a function of concentration, on ethylene evolution (Figure 5A) and pigment efflux (Figure 5B). For ethylene evolution, a biphasic response was observed for those surfactants with a POE content of 6 to 12 (Figure 5A). At low concentrations (0.01 to 0.05%), ethylene evolution was significantly greater

than in the controls. However, as the concentration increased above 0.05% (to 1.0%), ethylene evolution decreased substantially, with the largest decline being observed for those surfactants with the lowest POE content. In contrast to the other members of the Neodol series, Neodol 91-20 did not demonstrate a biphasic response. Instead, ethylene evolution continued to increase with increasing surfactant concentration.

No significant pigment efflux occurred with the Neodol 91 surfactants at concentrations less than 0.05% (Figure 5B). At concentrations above 0.05%, pigment efflux increased with an increase in concentration except with 91-20, which had no effect over the entire concentration range studied. Efflux was, in general, inversely related to POE content. If one plotted efflux vs. POE content for the 0.1% data, a reasonable linear relationship would be obtained. However, if the 1.0% data were utilized, the relationship between POE content and pigment efflux would not be clear. The reason for a greater response from Neodol 91-10 than from 91-8 at higher concentrations is not clear.

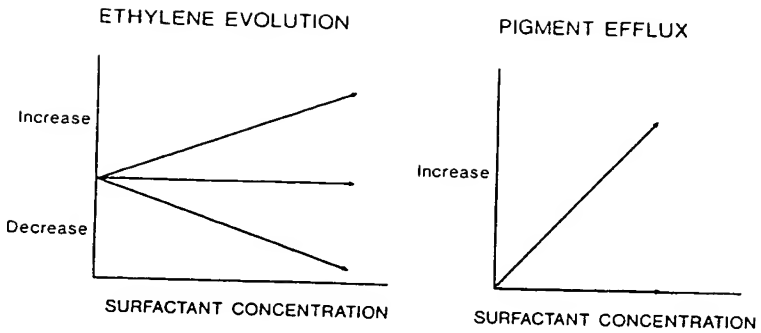
The log cmc values for the Neodol 91 series (Table 1) are between -1.6 and -1.4. Therefore, as can be seen from Figure 5, maximal stimulation of ethylene evolution for those Neodol surfactants with a POE content of between 6 and 12 occurs around the cmc. This corresponds to the point at which pigment efflux begins. As surfactant concentration increases above the cmc, ethylene evolution decreases while pigment efflux increases, suggesting that both responses are affected via surfactant action on membranes. This is consistent with the observations of others[1,2,24] that membrane (e.g., tonoplast) integrity is required for EFE activity.

Hydrophobe effects on ethylene evolution and pigment efflux are presented in Tables 2 and 3, respectively. For the two alkyl (linear alcohol) surfactants, maximal stimulation of ethylene evolution with the $C_{9\text{-}11}$ hydrophobe mixture was reached at 0.05%, compared to 0.5% for the $C_{12\text{-}15}$ hydrophobe mixture (Table 2). Of the two aryl hydrophobes, nonylphenol caused greater ethylene evolution across the entire concentration gradient examined. In terms of pigment efflux, the smaller hydrophobes ($C_{9\text{-}11}$ vs. $C_{12\text{-}15}$; octylphenol vs. nonylphenol) caused greater efflux (Table 3).

These data suggest that simply increasing the lipophilicity of the hydrophobe does not lead to increased biological activity. Indeed, the so-called "Ferguson effect" states that for maximum activity, a balance between lipophilicity and water solubility must be achieved.[38] Factors such as molecular weight, physical size, and the chemical nature and structure of the hydrophobe must also be considered. The importance of the latter point is underscored by the work of Siegel and Halpern,[40] who reported a decrease in alcohol-induced pigment efflux from beet root tissue when the alcohols were branched at the C-1 position.

Hydrophile:lipophile balance (HLB) is commonly used to categorize nonionic surfactants.[4] For polyethoxylated surfactants, HLB values are closely related to POE content. HLB values, in theory, range from 0 (least hydrophilic) to 20 (most hydrophilic). Although HLB value calculations do not factor in concentration (a particular concern for these studies, given the concentration-dependent nature of the ethylene evolution response) or chemical structure, this method of data presentation is useful because it helps visualize relationships between surfactant polarity and biological activity. We selected the Triton X series to demonstrate the relationship between HLB values and ethylene evolution and pigment efflux because of the wide polarity range within this series (Table 1).

At 0.1%, greater ethylene evolution was induced by surfactants having HLB values of 13 to 15 (Figure 6). When the data obtained for these same surfactants at 0.5% were plotted, no qualitative changes were observed. However, when the 1.0% data were utilized, the trend between HLB and maximal ethylene evolution was not readily apparent. For pigment efflux, we selected those data obtained with 1.0% surfactant for graphic presentation (Figure 6). Unlike the ethylene evolution data, pigment efflux for the Triton X series was linearly related to concentration. Therefore, the relationship between HLB and pigment efflux would not



RELATIONSHIP BETWEEN ETHYLENE EVOLUTION AND PIGMENT EFFLUX

Ethylene / Pigment Efflux	Increase	No Effect	Decrease
Increase	1,2,9,12,13 14,16,17,18, 19,21,22,23, 24,25,27, 28,29		6,7,8,10,11
No Effect	3,4,15,20,26	5	

FIGURE 7. Schematic illustration summarizing the relationships between ethylene evolution and pigment efflux for the 29 surfactants examined (see Table 1 for listing of numeric code). Categorization based on response observed at 0.1% surfactant.

change qualitatively if data from another surfactant concentration were used. Marked pigment efflux was induced by Triton X-100 (HLB value of 13.5). Buchanan[5] also found that within a series of Triton X surfactants, maximum pigment efflux from beet root tissue was induced with Triton X-100.

It is interesting to compare the HLB values which correspond to the maximum activity in our studies (13 to 15) with those reported by others. Egan et al.[9] observed maximum protein and lipid extraction from mitochondrial membranes at HLB values of 12.5 to 13.5 for a series of Triton X surfactants. As reviewed by Helenius and Simons,[16] studies on the solubilization of microsomes and viral and bacterial membranes have shown that maximum activity is achieved with surfactants whose HLB values are in the range of 12.5 to 14.5. Based on these reports, as well as the data reported herein, maximum membrane interaction appears to occur with surfactants having HLB values in the 12 to 15 range.

Earlier, the surfactants were categorized according to their concentration-dependent ethylene evolution and pigment efflux patterns. Of equal importance, however, are the relationships between ethylene evolution and pigment efflux for each of the surfactants at a given concentration. For this purpose, we compared surfactant data obtained at the 0.1% concentration. We chose this concentration because it represents a common field-use rate for spray applications. As previously mentioned, this type of categorization does not consider concentration-dependent behavior (e.g., biphasic responses).

To summarize, three types of surfactant effects on ethylene evolution were observed: an increase, an inhibition, or no effect (Figure 7). Likewise, with pigment efflux, two types

of surfactant effects were found: an increase or no effect. By creating a matrix of ethylene evolution and pigment efflux response categories, interrelationships between surfactant chemistry and biological activity can be visualized (Figure 7). Clearly, most (18 of 29) of the surfactants increased both ethylene evolution and pigment efflux. All but one were nonionic (Arquad 2C-75 is cationic). In terms of characteristics such as HLB, molecular weight, and cmc (Table 1), members of this group represented a relatively wide spectrum.

Five surfactants (Triton X-305, Triton X-405, Tween 20, Neodol 91-20, and Neodol 25-30) increased ethylene evolution without increasing pigment efflux (i.e., disrupting membrane integrity). All five were nonionic and have relatively high POE contents. One might speculate, as others[11,16,42] have, that surfactants possessing large molecular weights and/or sizes have a reduced level of interaction with cellular membranes, leading to diminished affinity (hydrophobic bonding) and depth of membrane penetration. Curiously, the increase in molecular weight and/or size led to a reduction in membrane disruption (i.e., pigment efflux) but did not diminish the ethylene response.

One surfactant, Tween 80, had no effect on either ethylene evolution or pigment efflux. If one compares Tween 20 with Tween 80 (Table 1), it appears that increasing the hydrophobe chain length from C_{12} to C_{18} reduces (eliminates?) surfactant interaction with beet root cell membranes. Sutton and Foy[45] also observed that Tween 20 induced greater pigment efflux from beet root tissue, compared to Tween 80. In terms of the relationship(s) between HLB values and biological activity, these results with Tween 80 are in contrast with those previously discussed. Surfactants with an HLB value of between 12 and 15 were identified as being the most active in terms of membrane interactions. Since Tween 80 has an HLB value of 15.0 (Table 1), this categorization clearly has limitations.

Five surfactants (Duponol, Aerosol OT, Aerosol OT-B, Arquad C-50, and Neodol 91-6) decreased ethylene evolution and increased pigment efflux. Three ionogenic classes of surfactant chemistry are represented by these five compounds, making it difficult to draw conclusions about surfactant chemistry/biological activity profiles. All five surfactants have relatively low molecular weights and would be expected to more readily penetrate into cell membranes. However, this is not the sole factor involved, since other surfactants with similar molecular weights (e.g., Neodol 25-3, Triton X-15) demonstrated different types of behavior (e.g., increased ethylene evolution and pigment efflux).

The empty boxes in Figure 7 also offer useful information. No surfactants increased pigment efflux without affecting ethylene evolution. Similarly, no surfactants decreased ethylene evolution without increasing pigment efflux.

The results of our studies demonstrate that surfactants possess different types and levels of membrane-mediated biological activity. While our studies did not focus on elucidating the basis of surfactant action on plant cell membranes, several observations and points of speculation deserve comment. The mechanism of surfactant action on ethylene evolution (EFE activity) is not clear. As reviewed by Helenius and Simons,[16] low concentrations of surfactant affect (i.e., inhibit, activate, or modify) most membrane-bound enzymes. Interestingly, some membrane enzymes which are activated by low concentrations of surfactant are also inhibited at higher concentrations of the same surfactant. If EFE responded to surfactants in this manner, it could explain some of our ethylene evolution results (e.g., Triton X-100, Figure 4B).

It is generally believed that the surfactant monomer, rather than the micelle, binds/sorbs to proteins.[16] This is particularly true for charged surfactants, where the nature of the charged head group and the length of the alkyl chain are important factors determining the degree of cooperative binding and subsequent conformational (denaturation) change(s). Nonionic surfactants typically do not induce cooperative binding, and therefore are less likely to denature proteins.

Binding/sorption of surfactant by proteins will compete with self-association processes (i.e., micelle formation) as the surfactant concentration reaches/exceeds the cmc.[16] Some insight into surfactant affinity for proteins may therefore be obtained by examining the relationship between surfactant concentration and ethylene evolution. For example, the anionic surfactant Duponol (Figure 4E) significantly inhibited ethylene evolution at pre-cmc concentrations, whereas the cationic surfactant Arquad C-50 (Figure 4F) had little effect on ethylene evolution until the cmc was exceeded. These results suggest that Duponol monomers may have a greater affinity for EFE, compared to Arquad C-50 monomers. This is interesting because cationic surfactants are typically considered more phytotoxic to plant tissues than anionic (or nonionic) surfactants.[13,23] Further detailed binding studies are required to elucidate this point.

It should be noted that in addition to a direct effect on the EFE system, surfactants may affect enzyme activity indirectly.[16] For example, the presence of monomers in the lipoidal membrane could affect membrane fluidity/permeability, leading to changes in the availability of substrate (ACC), cofactor(s), and/or inhibitor(s) for the enzymatic reaction(s). Likewise, the presence of micelles in the incubation medium may also affect assay results, due to micellarization (solubilization) of substrate, cofactor(s), and/or inhibitor(s).

In contrast to ethylene evolution, surfactant-induced cellular lysis and subsequent pigment efflux appears to be a relatively straightforward process.[16] Lysis can be divided into five stages: (1) surfactant monomers adsorb to the membrane, (2) the monomers penetrate into the membrane, (3) the monomers disrupt the molecular organization of the membrane, (4) membrane permeabilty increases, and (5) efflux of cellular contents begins. Since most of the pigment is located in the vacuole, the "membrane" mentioned above (items 1–4) refers to both the plasma membrane and the tonoplast. Each membrane, depending on composition, may well respond to a given surfactant monomer differently.

In conclusion, the beet root assay allows for the simultaneous measurement of surfactant-induced changes in two distinct membrane-associated phenomena. As was clearly evident by the different response patterns of ethylene evolution and pigment efflux, this approach of simultaneously monitoring more than one membrane-associated parameter has merit for a program designed to study the biological activity of surfactants. While several of the general relationships reported herein between surfactant chemistry and biological activity have been observed previously, our data revealed new information on the relationship between ethylene evolution and pigment efflux as related to surfactant/membrane interactions.

ACKNOWLEDGMENTS

The authors thank R. G. Fader for technical assistance in preparing the computer graphics for this paper and A. Heredia for determining the cmc for Triton N-42 and Tween 80. This paper is based on work supported in part by the Michigan Agricultural Experiment Station, the United States Department of Agriculture/Agricultural Research Service under SCA 58-5114-7-1002, and by a grant from the Shell Development Company.

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Chapter 5

INFLUENCE OF TWO POLYMERIC ADJUVANTS ON BIOAVAILABILITY OF GLYPHOSATE IN VISION® FORMULATION: RELEVANCE TO RAINWASHING OF DEPOSITS FROM FOLIAR SURFACES

Alam Sundaram

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ABSTRACT

The influence of two polymeric adjuvants, Sta-Put® and Silwet® Y-6652, on glyphosate washoff from trembling aspen foliage was studied using Vision® formulation at a dosage rate of 0.356 kg of active ingredient (ai) in 25 l/ha. End-use formulations were prepared by adding adjuvant concentration levels ranging from 0.05 to 1.5% v/v to diluted Vision® formulation containing ^{14}C-glyphosate. Two types of studies were carried out: one for investigating the rate of uptake, foliar washoff, translocation, and bioavailability of glyphosate; and the other for optimizing adjuvant concentration levels to maximize foliar uptake and to minimize washoff, without causing reduction in translocation and bioavailability. Young seedlings were used for the first study using end-use formulations containing 0.05% of the adjuvants. The data indicated that foliar uptake of glyphosate is a slow process since the amount washed off the foliage was >67% at 8 h after treatment and >50% at 48 h. Similar to the foliar uptake, translocation also occurred slowly, only about 2% of the applied amount was translocated in 8 h, and about 10% in 48 h. Nevertheless, there was no evidence of reduced bioavailability because of the presence of the adjuvants at the concentration level used.

Branch tips were used in the second study for treatment of end-use formulations containing 0.05 to 1.5% of the adjuvants, and were harvested at only one time period (48 h). The data indicated that both adjuvants contributed to reduced glyphosate washoff even at the low concentration level of 0.05%. The amount washed off decreased progressively as the adjuvant concentration increased from 0.05 to 1.5%. However, the amount that translocated into the untreated parts of the branch increased initially, passed through a maximum at an adjuvant concentration range of 0.3 to 0.6%, and decreased gradually at higher concentrations of 1.0 to 1.5%. The data thus indicated an optimum adjuvant concentration range beyond which reduced translocation and bioavailability would likely occur. Thus, the study indicated the need to optimize adjuvant concentration levels for every application condition. Otherwise, the rate of uptake and translocation is likely to be impaired, resulting in reduced herbicidal effectiveness.

I. INTRODUCTION

Rainfall after treatment has been shown to reduce the efficacy of postemergence herbicides.[1,2,4,5,7,9,15] The rain-free period after treatment, required to achieve adequate weed control, varied greatly, depending on the type of formulation.[12] The addition of some polymeric adjuvants has proved to be useful in protecting foliar deposits against washoff[17] and in improving the rainfastness of pesticides.[14] However, certain polymers can cause adverse side effects, such as reduced bioavailability via entrapment of the herbicide molecules in the polymeric structure.[8]

In the present study, aspects of foliar uptake and translocation into a forestry weed species were investigated as a means of assessing the bioavailability of radiolabeled glyphosate (*N*-(phosphonomethyl)glycine, Monsanto Agricultural Products Company, St. Louis, MO). Two polymeric adjuvants, Sta-Put® (Nalco Chemical Company, Naperville, IL) and Silwet® Y-6652 (Union Carbide, Danbury, CT), were investigated at different concentration levels in Vision® (a commercial formulation of glyphosate from Monsanto) formulation. The objective was to optimize the adjuvant concentration that would provide maximum protection against washoff with minimum reduction in bioavailability.

TABLE 1
Percentage Compositions of Ingredients in the Glyphosate Formulations Used in the Preliminary Investigations and in Study 1 of the Detailed Investigation

Formulation ingredients	Percentage composition (v/v)	
	Formulation VW	Formulation VWSt-0.05[a] or VWSi-0.05
Distilled water	42.00	41.95
Vision® (356 g of glyphosate/l)	4.00	4.00[b]
^{14}C-glyphosate[c]	54.00	54.00
Adjuvant	—	0.05

[a] The formulation VWSt-0.05 contained the Sta-Put® adjuvant, whereas the VWSi-0.05 formulation contained the Silwet® Y-6652 adjuvant.

[b] At a concentration level of 4.00 g of glyphosate in 100 ml, the dosage rate is equivalent to 0.356 kg of the active ingredient in 25 l of the formulation per ha.

[c] The radiolabeled product had a specific activity of 10 μCi per milligram of glyphosate in 1.00 ml of solution.

II. PRELIMINARY INVESTIGATIONS

Preliminary investigations were carried out to examine the adhesive capacity of the Sta-Put and Silwet Y-6652 polymers for glyphosate deposits. ^{14}C-labeled glyphosate formulations with and without the adjuvants (Table 1) were applied in droplets (each 0.5 μl in volume or 1000 μm in diameter) to glass plate surfaces (7.5 × 5.0 cm), at the rate of 2 μl or four droplets per plate, containing ^{14}C-glyphosate equivalent to 6000 dpm per drop, using a precision microapplicator (Instrumentation Specialties Company, 4700 Superior, Lincoln, NB).

Four replicate treatments were made for each formulation listed in Table 1. The droplets were washed from the glass plates 48 h later using water (2 × 20 ml for each glass plate); the time duration for washing was maintained at 30 s for each wash (equivalent to 60 s for each plate). The wash liquid was collected in a 50-ml measuring cylinder and an aliquot of 4 ml was added to 16 ml of an aqueous scintillation cocktail (Scinti Verse™ II, SO-X-12, Scientific Company, NJ). The ^{14}C-activity was determined by a Beckman LS9000 liquid scintillation counter (LSC) with a built-in automatic external standardization to determine counting efficiency. The range of counting efficiency was 95 to 99%, and the data indicated that both polymers exhibited some degree of adhesive capacity for glyphosate on glass plates. For example, the glyphosate on plates treated with the VW formulation (Table 1) containing no adjuvant was completely washed off in the 40-ml aliquot of water, whereas only about 60% of the applied glyphosate was washed from plates treated with VWSt-0.05, which contained the Sta-Put adjuvant at 0.05% (v/v), and only about 50% was washed from plates treated with VWSi-0.05, which contained Silwet Y-6652. The study thus indicated that both polymers are capable of providing rain protection for glyphosate deposits on target foliage, although Silwet Y-6652 will provide greater rain protection than Sta-Put at equal concentration levels.

In view of these findings, detailed investigations were undertaken to study the effect of different concentrations of the two polymers in Vision formulation on the washoff of glyphosate from treated leaf surfaces of trembling aspen (*Populus tremuloides* Michx.) seedlings

at different time intervals after treatment. Simultaneously, the uptake and translocation of glyphosate into plants were also investigated in two types of studies. Study 1 examined the uptake and translocation into different parts of plants at three different time intervals (up to 48 h) after treatment, using the three formulations listed in Table 1. The objective was to assess the rate of uptake, translocation, and redistribution of glyphosate in different parts of plants with formulations containing the lowest concentration of the adjuvants. Study 2 investigated the uptake and translocation of glyphosate into aspen branch tips at 48 h after treatment, using formulations containing five different concentrations of the adjuvants. The objective was to determine the optimum concentration of adjuvant that would provide the maximum foliar uptake with minimum washoff, and with little reduction in the bioavailability (i.e., translocation into untreated parts of plants) of glyphosate.

III. MATERIALS AND METHODS

A. MATERIALS

1. Study 1 — Uptake, Translocation, and Redistribution

The three glyphosate formulations used in study 1 are listed in Table 1 along with the percentage compositions of the ingredients used with them. The ^{14}C-glyphosate was purchased from Amersham Corporation, Oakville, Ontario, Canada, and the radiolabeling was on the phosphonomethyl carbon.

2. Study 2 — Optimization of Adjuvant Concentration

Three of the 11 formulations used in study 2 were the same as those listed in Table 1. The remaining formulations contained the same amount of Vision (4.0 parts) and ^{14}C-glyphosate (54.0 parts) as those listed in Table 1, but the adjuvant concentration was increased to 0.3, 0.6, 1.0, and 1.5 parts, followed by a simultaneous reduction in water levels so that the final volume would still be maintained at 100 parts by volume. Accordingly, formulations containing 0.3% of the adjuvant were described as VWSt-0.3 or VWSi-0.3, those containing 0.6% as VWSt-0.6 or VWSi-0.6, etc.

B. METHODS

1. Study 1 — Uptake, Translocation, and Redistribution

The majority of the bioavailable portion of the applied herbicides is known[11,16] to be absorbed into plants within 48 h after treatment. Therefore, the absorption and translocation aspects were studied only up to 48 h.

2. Trembling Aspen Seedlings

Thirty trembling aspen seedlings were removed from the field in which they were grown when the seedling height was about 20 cm, planted in pots, placed in a greenhouse, and maintained under constant conditions of temperature (15 ± 1°C), photoperiod (16 h of light; 8 h of darkness), and relative humidity (75 ± 7%) for 4 weeks for acclimatization. At the time of the study, the mean ± SD of the height was 40 ± 5 cm (62 ± 5 cm including the pot), the maximum diameter was 16 ± 2 cm, and all the seedlings had 16 ± 3 leaves. The surface area of the leaves at the middle portion of the crown was 12.5 ± 2 cm^2 per leaf. Twenty-seven seedlings were divided into three groups (A, B and C), each consisting of nine seedlings. Group A received the VW formulation, group B, VWSt-0.05, and group C, VWSi-0.05, respectively. The remaining three seedlings served as controls for measuring the background radioactivity in the plants.

TABLE 2
Foliar Uptake and Translocation of Glyphosate in Vision® Formulation with and without Two Polymeric Adjuvants — Percentage Distribution of Radioactivity in the Different Samples Analyzed

Sample description	Percentage distribution*								
	VW			VWSt-0.05			VWSi-0.05		
	8 h	24 h	48 h	8 h	24 h	48 h	8 h	24 h	48 h
Treated leaves	21.69	27.55	28.35	30.79	35.55	34.23	29.89	36.55	36.75
Leaves above treated leaves	0.26	0.49	0.93	0.30	0.60	0.90	0.30	0.50	0.80
Leaves below treated leaves	0.24	0.55	1.01	0.24	0.70	1.21	0.40	0.60	1.30
Stem	0.54	1.11	3.77	0.50	1.20	4.20	0.60	1.10	4.30
Root	0.15	1.70	4.21	0.20	2.30	5.41	0.30	2.10	4.41
Plant total	22.88	31.40	38.27	32.03	40.35	45.95	31.49	40.85	47.56
Leaf wash	76.80	68.20	61.13	67.74	59.31	53.54	68.36	58.91	52.08
Soil extract	0.02	0.03	0.05	0.03	0.04	0.06	0.05	0.04	0.06
Grand total	99.70	99.63	99.45	99.80	99.70	99.55	99.90	99.80	99.70
Percent	0.30	0.37	0.55	0.20	0.30	0.45	0.10	0.20	0.30

* Values represent the mean of three sets of data obtained from three trees. All values were corrected for the ^{14}C counting efficiency.

3. Glyphosate Treatment

A 25-μl aliquot of the formulations listed in Table 1 (containing 0.135 μCi of ^{14}C-glyphosate or 300,000 dpm of radioactivity) was applied in 50 × 0.5 μl drops (1000 μm in diameter) to the middle portion of the crown of eight leaves of each plant at the rate of six drops per leaf for six leaves and seven drops per leaf for two leaves (to provide an average of 0.5 drops per cm^2), using the microapplicator described in Section II.

4. Sampling and Analysis

Of the nine seedlings used for each formulation, three were harvested at 8, 24, and 48 h, respectively. Each plant was divided into five segments: treated leaves (TL), leaves above treated leaves (LATL), stem (ST), leaves below treated leaves (LBTL), and root (RT). The TL samples were washed twice with 20 ml of distilled water (for 30 s each time), and the wash liquid (treated leaf wash, TLW) was assayed for ^{14}C activity as described in Section II. All plant parts, including the treated leaf residue (TLR), were then oven dried for 14 h at 60°C, weighed, and combusted in a biological sample oxidizer (Packard Oxidizer, Model 306, United Technologies Packard, Packard Instrument Company, Illinois). The evolved $^{14}CO_2$ activity was absorbed in vials containing Carbosorb® (an aqueous counting scintillant, United Technologies Packard) for the ^{14}C assay.

The wet soil from the pot was filtered under a vacuum to remove the water, and the residue was washed twice with 20 ml of distilled water. The washings were added to the filtrate, which was then concentrated to a final volume of 3 ml for the ^{14}C assay, as described above for the aqueous rinse of the TL samples.

The ^{14}C activity of all samples was determined using the Beckman LSC as described in Section II. The range of counting efficiency was 94 to 98%, and the data in Table 2 were corrected for this. Because few glyphosate metabolites have been reported in plants within 48 h after treatment,[6,10,18] the radioactivity recovered will be referred to as ^{14}C-glyphosate.

5. Study 2 — Optimization of Adjuvant Concentration

To investigate foliar uptake and translocation of glyphosate with several concentrations of the two adjuvants, a large number of seedlings would be required. However, the use of

TABLE 3
Foliar Uptake and Translocation of Glyphosate into Aspen Branch Tips at 48 h after Treatment with the Formulations, without and with Adjuvants, at Different Concentration Levels — Percentage Distribution of Radioactivity

Formulation description	Sample description			
	Treated leaf	Remaining parts	Treated leaf wash	Tap water in vial
VW	31.30[a]	4.54	64.10	0.06
VWSt-0.05	38.30	5.85	55.75	0.10
VWSt-0.30	42.80	5.85	51.27	0.08
VWSt-0.60	48.30	6.65	44.80	0.25
VWSt-1.00	52.00	3.70	44.20	0.10
VWSt-1.50	58.00	2.50	39.47	0.03
VWSi-0.05	38.55	5.10	56.27	0.08
VWSi-0.30	38.73	7.35	53.67	0.25
VWSi-0.60	42.74	5.10	52.01	0.15
VWSi-1.00	48.15	3.85	47.80	0.20
VWSi-1.50	55.93	2.85	41.02	0.20

[a] Values represent the mean ± SD calculated from six sets of data obtained from the six branch tips used for each formulation. All values were corrected for the ^{14}C counting efficiency. Percentage distribution values were calculated as:

$$\% \text{ Distribution} = \frac{\text{Radioactivity recovered in sample}}{\text{Total radioactivity recovered}} \times 100$$

a large number of seedlings would involve extensive labor, time, and cost of materials. To overcome this problem, small branch tips were used in study 2.

Sixty-eight branch tips (each 20 cm long, containing four fully developed young leaves) were clipped from the top portion of the seedlings (one branch from each seedling) which were maintained in the greenhouse, as described in study 1. The underdeveloped young leaves, except the shoots, were removed and discarded, leaving only the four fully developed leaves and shoots in the branch tip. The stem of each branch was immediately placed in a 50-ml plastic vial containing tap water, and the branch was supported upright by tubing and a lid with a hole. Similar branch clippings were tested for their survival rate and growth patterns for up to 7 d in a preliminary investigation prior to the actual study; the branches remained healthy, but showed a small reduction in weight during the first 2 d. However, weight gain was noted from the third day onward, and the plants grew quite well afterward.

Sixty-six branches were divided into 11 groups, G1 to G11, each consisting of six branches. Table 3 lists the different group numbers together with the formulations which were applied to each group. The remaining two branches (group G12) served as controls for measuring the background radioactivity in the branches. A 12-μl volume (containing 144,000 dpm of radioactivity) of each formulation was applied in 24 × 0.5 μl drops to four leaves (total surface area 50 cm^2) at the rate of six drops per leaf. For relevant details, see Section B.3

The six branches used for each formulation were harvested at 48-h posttreatment. This time period was considered adequate for detecting differences in the uptake and translocation patterns between the three formulations used. Each branch was divided into two parts: treated

leaf and the remaining parts. The tap water in the vial was also collected for radioassay to examine glyphosate movement via the stem into the water. The treated leaf was rinsed as described above to provide TLR and TLW. All samples were assayed for ^{14}C-glyphosate in the same manner as mentioned in Section B.4. These data are given in Table 3.

IV. RESULTS AND DISCUSSION

A. STUDY 1 — UPTAKE, TRANSLOCATION, AND REDISTRIBUTION

Data in Table 2 indicate that foliar uptake of glyphosate is a relatively slow process, since more than 67% was washed off into the leaf rinse at 8 h after treatment, irrespective of the type of formulation tested. However, as the exposure duration increased, the uptake gradually increased to 28 to 37%, depending on the adjuvant, and only about 52 to 61% was washed off at 48 h.

Similar to the foliar uptake, the translocation of glyphosate from the treated leaf into other parts of the seedlings was also slow, as only about 2% of the applied amount was translocated at 8 h after treatment, and approximately 98% remained in the treated leaf. However, with the increased exposure, translocation increased gradually and reached 10 to 11% at 48 h (Table 2). Nevertheless, the treated leaf still contained about 88 to 89% of the applied amount, thus indicating incomplete translocation even after 48 h. The amount of radioactivity detected in the stem and root sections increased gradually from the 8-h value, to about four to five times higher at 48 h. The present findings are in agreement with those reported in the literature,[11] although the amount absorbed and translocated was slightly higher in the present study than in those reported.

Regarding the influence of the two polymeric adjuvants on the bioavailability of glyphosate, the present data indicate no evidence of glyphosate entrapment in the polymeric chain, thereby making it less bioavailable. On the contrary, the two adjuvants contributed to a significant increase in foliar uptake, as indicated by an analysis of variance test (ANOVA $p \leq 0.05$), yet the translocated amount seemed to be similar for all three formulations. Consequently, it appears that the increase in foliar uptake does not necessarily indicate an increase in the penetration of glyphosate through the leaf cuticle, since the adjuvants could have simply provided a protective layer over the droplets, thus reducing the amount being washed off during rinsing. Without detailed investigations using extracted plant cuticle,[3] it would not be possible to determine whether the two polymers actually increased the foliar uptake of glyphosate or simply provided a protective film over the droplets. Nevertheless, the present study indicated no evidence of reduced bioavailability of glyphosate at an adjuvant concentration level of 0.05% v/v.

B. STUDY 2 — OPTIMIZATION OF ADJUVANT CONCENTRATION

The results obtained from the six branch tips used in study 2 for each formulation were subjected to statistical treatment using the Student-Newman-Keuls test (SNK).[13] The data indicated that, on average, about 64% of the applied amount was washed (at 48 h) from the leaf treated with VW, as opposed to about 56% with VWSt-0.05 and VWSi-0.05 (Table 3), thus indicating a significant decrease (SNK error rate $\alpha \leq 0.05$) in the "apparent foliar uptake" because of the presence of the two adjuvants. The amount translocated into the remaining parts of the branch tip was 4.54% for VW, but was slightly higher for VWSt-0.05 and VWSi-0.05. However, as the concentration of the adjuvants increased to 1.5%, the amount of glyphosate that was washed from the treated leaves decreased progressively, whereas the nonwashable residue in the treated leaves increased correspondingly. On the other hand, the amount that was translocated into the remaining parts (i.e., the stem and the new shoots) of the branch tips showed an increase up to an adjuvant level of 0.6%, but

decreased rapidly as the concentration level increased further to 1.5%. Thus, the data clearly indicate the role of adjuvant concentration on the foliar uptake, translocation, and bioavailability of glyphosate. It appears that there is an optimum level of the two adjuvants at which the translocation occurs to a maximum extent, beyond which the translocation process is likely impaired. The reason for this could be that glyphosate is adsorbed and/or trapped into the cross-linked polymeric chain at polymer concentrations greater than 0.6%. The present study demonstrated the need for optimizing the adjuvant concentration for every application condition, because the use of different volume rates would alter the concentration of the surfactant which is already present in the commercial formulation concentrate, and hence the adjuvant concentration would correspondingly require optimization, depending on the volume rate of application used.

V. CONCLUSIONS

The present findings demonstrated that both Sta-Put® and Silwet® Y-6652 show some potential for providing rainfastness for glyphosate deposits on foliage, since the amount of washable glyphosate was reduced in the presence of the two adjuvants, compared to the amount that was washed off without the adjuvants. Nevertheless, the need to optimize the concentration level of the adjuvants is indicated for every application condition. Otherwise, the active ingredient would likely be less bioavailable as a result of reduced translocation into the active sites of the plants, thus possibly resulting in impaired herbicidal effectiveness.

ACKNOWLEDGMENT

The author wishes to thank Mr. John W. Leung for his technical help in carrying out the numerous measurements required for this investigation.

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Chapter 6

A FOLIAR UPTAKE MODEL OF TRICLOPYR

Rosalind D. Buick, Roger J. Field, A. Bruce Robson, and Graeme D. Buchan

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ABSTRACT

Foliar uptake of a triethylamine salt of ^{14}C-triclopyr into field bean (*Vicia faba* L. cv. Maris Bead) leaves was enhanced by the addition of a nonionic organosilicone surfactant, Silwet® L77. The surfactant significantly reduced the surface tension of triclopyr solutions to 22 mN m^{-1}, although improved foliar uptake could not be solely attributed to surface tension changes. Fluorescence microscopy of herbicide solutions containing a fluorescent indicator showed that addition of Silwet L77 permitted these solutions to penetrate rapidly into the stomatal pores on the adaxial surface of the bean leaf.

A model of the uptake process for triclopyr and the modifications induced by Silwet L77 has been established. The model assists in determining the mode of action of Silwet L77 and can be used in interpreting the action of other organosilicone surfactants.

I. INTRODUCTION

The cuticle is the most significant barrier to herbicide absorption into shoot or foliar tissue.[13] Herbicides must penetrate the cuticle in order to gain entry to the plant. The potential pathways of herbicide absorption may be through the cuticle proper and/or through the stomata. If infiltration of active ingredient into substomatal cavities is appreciable, it would be advantageous since:

1. It would reduce the duration of the postspray rain-free of the herbicide.
2. Relatively humid conditions within the substomatal cavity would prolong the time the active ingredient is in solution and, therefore, can be absorbed and subsequently translocated.

Many researchers have shown herbicide entry via stomata to be important.[10,12,17,18] Schonherr and Bukovac[18] found that entry through stomatal pores depends on three main factors: (1) plant surface wettability, (2) stomatal morphology, and (3) solution surface tension. If the surface tension of the herbicide formulation is at or below the critical tension value for the solution and plant surface, instantaneous stomatal entry can occur. Critical surface tension values can be determined for each surface and solution combination.[19] Critical surface tension is defined as the surface tension at which the contact angle is 0° (i.e., cosine of contact angle $\theta_A = 1$). An idea of the major chemical components of the surface can also be deduced from these values.[19]

In aqueous systems such as those containing herbicides, the inherently high surface and interfacial tensions can be reduced by the addition of relatively small amounts of a surfactant.[15] Nonionic organosilicone surfactants are one surfactant group which have been observed to markedly reduce surface tension and enhance the activities of a number of herbicides.[14] The promotion of glyphosate efficacy was explained by the surfactant, Silwet L77, enabling stomatal infiltration of herbicide solutions to occur.[12]

In this study, foliar uptake of an amine salt triclopyr {[(3,5,6-trichloro-2-pyridinyl) oxy] acetic acid}, by field bean was investigated in combination with the nonionic organosilicone surfactant, Silwet L77. Fluorescence microscopy was used to visualize the effect of this surfactant on the foliar uptake process. Surface tension and contact angle relationships were also studied and related to the foliar uptake findings. A computer modeling approach is adopted to describe the triclopyr uptake process by field bean. Such an approach will help to quantify the important features by which Silwet L77 enhances uptake of the herbicide.

II. MATERIALS AND METHODS

A. PLANT MATERIALS AND GROWTH CONDITIONS

Field bean seed was pregerminated and potted into 100-mm pots containing a potting mix. Plants were grown in a controlled environment cabinet with the following conditions; day/night temperature 20 to 23/15°C, 15-h photoperiod, 70 to 75% relative humidity, PPFD of 300 μmol m^{-2} s^{-1}, and a daily watering regime. All experimental procedures with plants were carried out on adaxial leaf surfaces of the third leaves once they had fully expanded.

B. FOLIAR UPTAKE STUDY

Solutions of ^{14}C-triclopyr triethylamine were made by adding ^{14}C-triclopyr acid to a solution of triclopyr amine (360 g l^{-1} triclopyr acid as the triethylamine salt) made up to a field equivalent rate of 4 kg of active ingredient (a.i. per hectare in 200 l ha^{-1}). Concentrations of labeled and nonlabeled triclopyr were such that 1 μl of herbicide solution yielded 20,000 to 30,000 dpm (approximately 0.01 μCi). Silwet L77 was included at a rate of 0.25% (v/v) in the surfactant treatments. Each plant received 1 μl as four 0.25-μl droplets 2 h after the beginning of the photoperiod. Eight replicate plants per treatment were used.

At various time intervals over a 2-h period, leaf surfaces were washed with 70% methanol into 25-ml volumetric flasks. The percentage of gross uptake was determined by dividing the radioactivity in the methanol wash by the total radioactivity applied. Immediately after the methanol wash, a second leaf wash with 10 ml of chloroform was used to determine the amount of radiolabel in the wax as a percentage of the total applied radioactivity. The percentage net uptake of triclopyr was determined by subtracting the wax uptake value from the gross uptake (i.e., the amount of triclopyr that entered the plant symplast or apoplast). All radiolabeled solutions were counted in 10 ml of Bray's scintillation fluid by a liquid scintillation counter. The results for the foliar uptake of triclopyr into field bean were analyzed as a function of the time from droplet application. A model of triclopyr uptake was proposed from the results.

C. SURFACE TENSION

Solutions of triclopyr amine were made up in deionized water to a field equivalent rate of 4 kg a.i. ha^{-1} in 200 l ha^{-1}. Various concentrations of Silwet L77 were added to these amine solutions: 0, 0.005, 0.01, 0.05, 0.1, 0.25, and 0.5% (v/v). All measurement conditions and solutions were held at a constant temperature of 22°C.

Surface tension was measured using an adapted droplet-volume technique described by Taylor.[21] The droplet volumes for each solution were measured by noting the change in the micrometer readings. Clamp stands and weights were used to eliminate vibrations during droplet formation. Using a conversion table of F values,[1] surface tension could be determined on the basis of the droplet volume and the precise diameter of the needle being used. (External needle diameters in these studies were always 2.01 mm, as measured by a traveling microscope.) Twenty replicate droplets were formed from the apparatus for each solution.

D. CONTACT ANGLES θ_A

The advancing contact angles (θ_A) of herbicide droplets on the adaxial surfaces of bean leaves were recorded using a horizontally oriented traveling microscope. All droplets were 1 μl in size and positioned 5 mm away from the leaf midrib. Solutions of triclopyr amine with or without Silwet L77 were identical to the concentrations used in surface tension measurements. Ten replicate droplets per solution were formed. Temperature and humidity were maintained at 22°C and 70 to 75%, respectively. Advancing contact angles were determined using Mack's[16] formula [$\theta_A = 2 \tan^{-1} (h|r)$], in which the droplet radius (r) and

maximum height (h) were measured with an eyepiece graticule in the microscope. Droplet heights and radii were always recorded 10 to 20 s after the drops were applied to the leaf.

Cosines of the contact angles were plotted against surface tension values for each solution to obtain a Zisman plot.[19] The critical surface tension value for the field bean adaxial leaf surface could then be determined by extrapolating a line of best fit for the plotted data, back to where cosine $\theta_A = 1$.

E. FLUORESCENCE MICROSCOPY

Solutions of triclopyr amine with Silwet L77 at 0, 0.10, 0.25, or 0.50% (v/v) plus Uvitex 2B, a fluorescent indicator (Ciba-Geigy), at 1 g l^{-1} were prepared. Bean leaves were treated with four 0.5-µl droplets, 5 to 10 mm away from the leaf midrib. At selected times (60 s and 1 h), treated leaves were first rinsed with deionized water, then with 70% methanol. The two rinses were carried out to ensure that no herbicide residue remained on the leaf surface. Rinsed leaves were gently wiped with clean tissue and mounted onto glass slides for observation. Transverse sections through randomly selected, treated leaves were also prepared to visualize the extent of herbicide penetration into the leaf. Herbicide solutions were detected using a Zeiss fluorescence microscope with a UV excitation source (Exciter filter G365, Chromatic beam splitter FT420, Barrier filter LP418). Photomicrographs were obtained using Kodak color negative film (Ektachrome 100 Professional). Five to seven photographs were taken per treatment to capture representative regions of herbicide absorption within the droplet area.

Image analysis was then used to quantify the mean areas of herbicide absorption in each photomicrograph, as shown by the fluorescent indicator. The system was an image analysis package called PC-Semper (Synoptics Ltd., Cambridge, U.K.), with attached camera and video monitors, capable of scanning images of up to 512 × 512 pixels with 256 grey levels. Threshold intensities were selected to obtain a scanned video image as close to the micrograph as possible. The mean values for degree of herbicide absorption were expressed for each treatment as:

1. Area of absorption as a percent of the whole photomicrograph area
2. Absorption area (mm^2) in the whole photomicrograph area

Transformations were performed on the data to validate the ANOVA assumption of equal variances. A square-root transformation of the area (mm^2) and a logit transformation on the percentage area were the most suitable. (The logit transformation was as follows: $y = \log_{10} [x|(100 - x)^b)$. As statistical inferences were made on the transformed data, standard errors of the transformed means are given.

III. RESULTS

A. SURFACE TENSION

The surface tension of the triclopyr amine solution with no added surfactant was 53.4 ± 1.42 mN m^{-1}, but this was significantly reduced by the addition of Silwet L77 (Figure 1). At concentrations greater than 0.05% (v/v), there were no significant changes in surface tension from a mean of 22.8 mN m^{-1}. Thus, the largest decrease in surface tension occurred with the addition of up to 0.05% (v/v) Silwet L77.

B. CONTACT ANGLE AND ZISMAN PLOT

A quadratic curve gave the best fit to the relationship between surface tension and the cosine of the contact angle; $R^2 = 83\%$ (Figure 2). The equation is as follows:

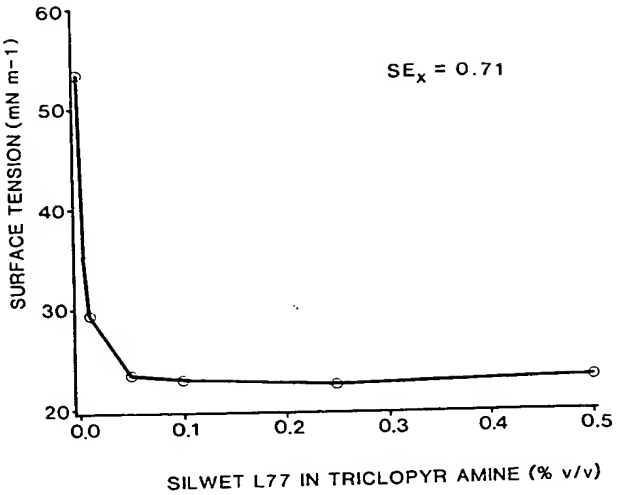

FIGURE 1. Surface tension (mN m^{-1}) of triclopyr triethylamine formulation plus Silwet® L77 surfactant (0 to 0.5%, v/v). A pooled standard error (SE_x) of the mean is given.

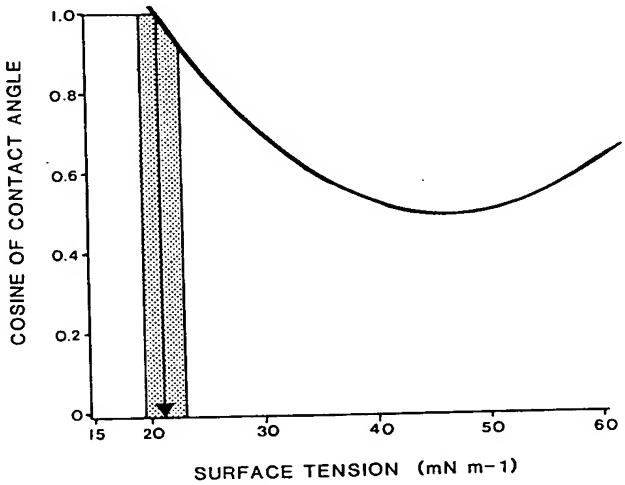

FIGURE 2. The quadratic relationship between surface tension of the triclopyr triethylamine formulation and cosine of the contact angles of droplets placed on the adaxial surface. Mean critical surface tension (γ_{crit}) value for this surface (when cosine θ_A = 1.0) was 21.11 mN m^{-1} with 95% confidence limits shown.

$$y = 2.09 - 0.0655x + 0.000667x^2$$

where x = the surface tension in mN m^{-1} and y = the cosine of the contact angle (θ_A).

From this, the critical surface tension value (γ_{crit}) for the adaxial leaf surface of field bean wax calculated as 21.11 mN m^{-1}. Parallel curves fitted to provide a 95% confidence limit yielded a γ_{crit} range of 19.45 to 22.87 mN m^{-1}.

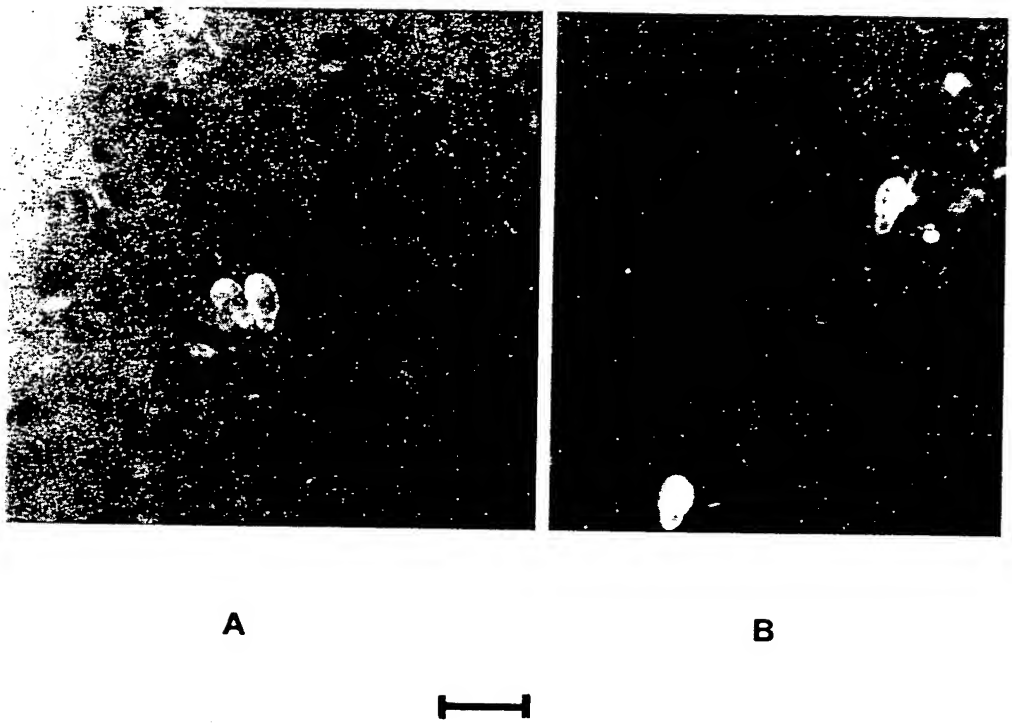

FIGURE 3. Photomicrographs showing typical areas of herbicide absorption after triclopyr amine control solutions + Uvitex 2B indicator were applied to leaves for 60 s (A) or 1 h (B). Glandular trichomes (hairs) and dust particles were the only regions to absorb the formulation. Scale = 100 μm.

C. FLUORESCENCE STUDIES

After both 60-s and 1-h washes, the control herbicide solutions (i.e., no added Silwet L77) had not penetrated the adaxial surface of field bean leaves, as shown by the relative absence of fluorescent dye across their surface (Figure 3). The glandular hairs or trichomes and any dust particles on the leaves were the only parts which appeared to absorb the fluorescent indicator and presumably the herbicide. Transverse sections through leaves also indicated no herbicide penetration into the leaf.

When Silwet L77 was added to the triclopyr amine at 0.1% (v/v), there were some small and infrequent regions of herbicide absorption apparent after 1 h (Figure 4), but nothing after 60 s. Silwet L77 at 0.25 or 0.5% (v/v) were similar, inducing extensive regions of herbicide uptake, after both 60 s and 1 h. Figure 5 shows this for the 0.25% (v/v) Silwet L77 treatment. Herbicide damage to the leaf epidermal cells was often visible after 1 h with these two Silwet L77 rates. This was characterized by large, brightly lit regions of indicator completely covering the site of droplet application (Figure 5C). Transverse sections through the leaf also displayed considerable penetration of fluorescent labeled triclopyr amine with these treatments after both 60 s and 1 h.

Image analysis showed that the mean area of triclopyr absorption was always greater after 1 h than after 60 s within all treatments. The average percentage absorbed for 60-s and 1-h treatments were 6.0 and 18.9%, respectively. This difference between the times was most notable for 0.25 and 0.5% (v/v) Silwet L77 treatments (Table 1). This was also depicted in the photomicrographs.

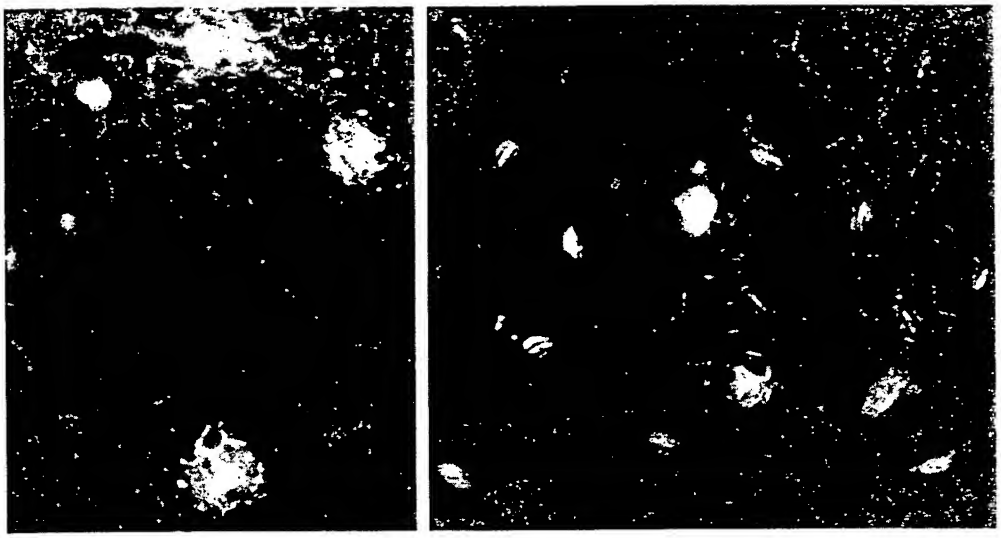

FIGURE 4. Photomicrographs showing typical areas of herbicide absorption after triclopyr amine solutions + 0.1% (v/v) Silwet® L77 + Uvitex 2B indicator were applied to leaves for 1 h. Glandular trichomes (arrow) and some small regions associated with stomatal pores absorbed the formulation. Scale = 100 μm.

Herbicide absorption areas were significantly greater when 0.25 and 0.50% (v/v) rates of Silwet L77 were added to the amine formulation. These two treatments were not significantly different from each other (Table 1). Similarly, the control (0%, v/v) and 0.1% Silwet L77 treatments were not significantly different from each other. The interaction between time and solution treatments was not significant.

A range of herbicide absorption patterns was often detected for a single treatment (Figure 5). This was reflected in the large standard errors for some treatments in Table 1. Table 1 provides the transformed means and standard errors of the means on which statistical inferences were based.

D. FOLIAR UPTAKE STUDY

During the 2-h uptake study, Silwet L77 at 0.25% v/v significantly enhanced the foliar penetration of ^{14}C-triclopyr amine into the adaxial surface of the field bean leaf (Figure 6). The gross and net uptake of solutions without Silwet L77 were significantly less than with Silwet L77 at all stages. A logarithmic transformation was performed on the raw data to obtain uniform errors among the treatments. Linear regressions were then used to describe each treatment (control and Silwet L77) in terms of time. Ninety five percent confidence limits on the two lines showed these to be significantly different.

Wax uptake was always small: less than 1% of applied triclopyr for the control and less than 2% for Silwet L77. Silwet L77-treated leaves tended to have higher wax uptake than the control, although this was only significant after 60 min. Relatively small wax uptake values resulted in net uptake following the same trends as gross uptake.

The mean observed uptake data were used to propose a model of triclopyr uptake (Figure 6). The initial triclopyr uptake rate for the control (i.e., up to 60 min.) was markedly slower than that for the Silwet L77 treatment. During the latter stages (i.e., 60 to 120 min), the rates of uptake for both treatments were similar, as depicted by the approximately parallel

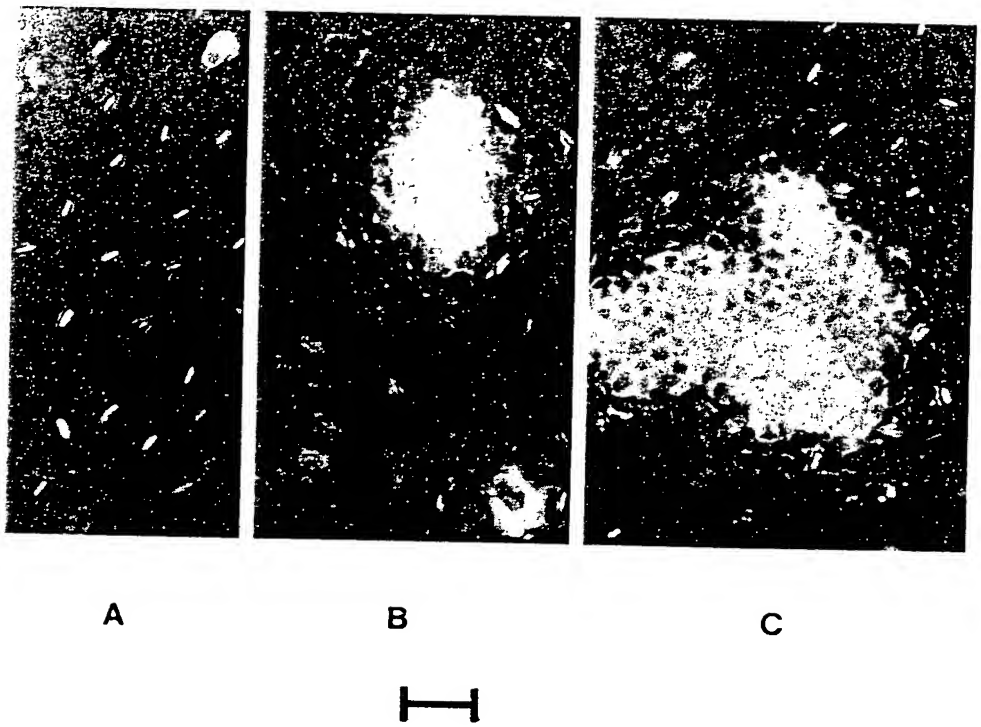

FIGURE 5. Photomicrographs showing the range in typical areas of herbicide absorption after triclopyr amine solutions + 0.25% (v/v) Silwet® L77 + Uvitex 2B indicator were applied to leaves for 60 s. Glandular trichomes had absorbed the formulation and extensive regions of absorption were associated with stomatal pores. Scale = 100 μm.

lines in Figure 6. The biphasic or two-stage uptake suggested by these results are discussed in terms of the possible routes of foliar penetration.

IV. DISCUSSION

Surfactants are included in herbicide formulations to enhance the retention and penetration of the active ingredient.[4] Nonionic organosilicone surfactants belong to a group known for their excellent wetting properties,[2] of which Silwet L77 is one. Several researchers have proposed that Silwet L77 promotes entry of herbicide solutions via stomatal pores.[7,12,22] Most surfactants do not appear to act by promoting translocation of herbicides per se.[3] Studies with Silwet L77 also suggest that it probably does not enhance efficacy by improving herbicide translocation.[6,9] Whether the promotion of uptake due to Silwet L77 can be totally accounted for by stomatal infiltration is not known.

A significant reduction in surface tension of the triclopyr amine formulation was observed when very small concentrations of Silwet L77 were added (Figure 1). The greatest drop in surface tension occurred up to 0.05% (v/v) Silwet L77. Addition above 0.05% (v/v) did not significantly alter the surface tension, i.e., a plateau surface tension was reached of approximately 23 mN m^{-1}. This phenomenon is typical of many surfactants in aqueous systems.[4,15]

TABLE 1
Image Analysis Results from Fluorescence Photomicrographs

Treatment of Silwet L77 (% v/v)	Time	Mean absorption area as % of total area	Mean absorption area (mm^2)
Control (0% v/v)	60 s	0.94	0.19
		(−2.02, 0.52)	(0.43, 0.75)
	1 h	1.00	0.20
		(−2.07, 0.30)	(0.43, 0.43)
0.1% Silwet	60 s	0.52	0.10
		(−2.30, 0.36)	(0.32, 0.53)
	1 h	1.57	0.31
		(−1.81, 0.26)	(0.56, 0.37)
0.25% Silwet	60 s	8.26	1.65
		(−1.18, 0.19)	(1.19, 0.28)
	1 h	39.28	7.78
		(−0.43, 0.19)	(2.50, 0.28)
0.5% Silwet	60 s	12.65	2.53
		(−0.86, 0.23)	(1.57, 0.33)
	1 h	33.62	6.73
		(−0.32, 0.30)	(2.55, 0.43)

Note: Values are means for individual treatments. Values in parentheses are transformed mean percentage absorption (logit) or transformed mean absorption area (square root), followed by standard errors of the transformed means.

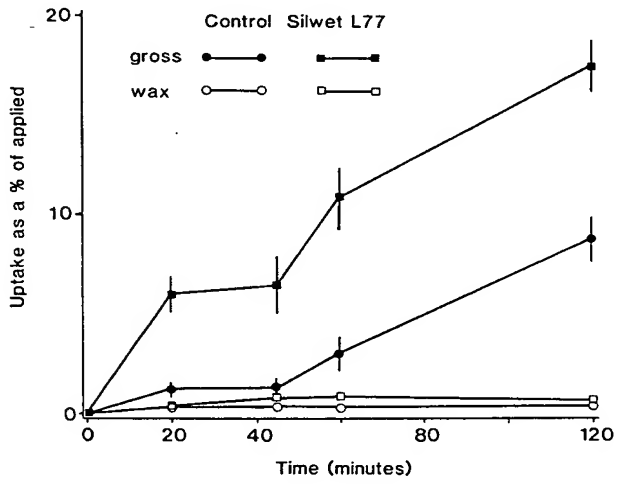

FIGURE 6. The effect of Silwet® L77 (0.25%, v/v) on gross and wax uptake of ^{14}C-triclopyr triethylamine (expressed as percent of total applied triclopyr). Standard errors of the means are shown as bars on either side of the plotted points.

Droplet contact angles can provide an index of the degree of surface wetting and spreading obtained by that solution on a specific plant surface.[17] As leaf surfaces are generally water repellent (hydrophobic) due to a thin coating of wax over the epidermis, the addition of a surface-active agent (surfactant) can improve the retention of spray droplets by operating at the interface between hydrophilic (droplet) and hydrophobic (plant) phases. In this study,

the cosine of the contact angle on adaxial bean leaf surfaces was plotted against surface tension to produce a Zisman plot. This relationship could then be used to determine the critical surface tension value for that surface and solution, i.e., the surface tension at which the cosine of the contact angle is 1.0 (or $\theta_A = 0°$).[19] For the adaxial leaf surface of field bean, the critical surface tension was 19.5 to 22.9 mN m^{-1} (mean of 21.11 mN m^{-1}). This was calculated by equating the derived version of the fitted quadratic curve to 1.0. Schonherr and Bukovac[10] obtained a value of 25 to 30 mN m^{-1} for the lower (abaxial) leaf surfaces of *Zebrina purpusii* Bruckn. Their critical value may be slightly higher than that for field bean because of differences in the chemical composition and spatial arrangement of the wax constituents.[9,11,19] The surfactant solution studies by Schonherr and Bukovac[18] did not contain triclopyr amine and Silwet L77, which would contribute to a different critical tension value. A tendency of the triclopyr formulations to dissolve the surface molecules of field bean may also explain the lower critical tension value.[11] Scanning electron microscopy supports a surface disruption by triclopyr.[8]

Zisman plots typically form a rectilinear relation between the cosine of the contact angle and the surface tension for a solution series.[19] Determination of the critical surface tension of liquids from such plots has proved useful in describing the spreading behavior of various liquids on a surface. Critical values have also been related to the constitution of various low-energy surfaces. As found with field bean, a critical surface tension of 19.5 to 22.9 mN m^{-1} on a hydrocarbon surface is a low value and is typically found with surfaces comprised of closely packed methyl groups.[19]

Although this rectilinear relationship is true for most solutions on most surfaces, the data band may exhibit curvature for values above 50 mN m^{-1} on some low-energy surfaces.[19] This phenomenon is believed to be due to weak hydrogen bonds forming between the molecules of liquid and those on the solid surface. This was observed in the studies with adaxial leaf surfaces of field bean and was consistent with the observation that the quadratic curve best described the relationship (Figure 2). Up to 45 to 50 mN m^{-1}, the points on the Zisman plot formed a linear band, but above this surface tension, the points tended to curve back up. It has been shown that such curvature is a result of weak hydrogen bonds forming between the molecules of liquid and those in the solid surface.[11] However, attaching importance to the quadratic curve in the region over 45 mN m^{-1} should not be done due to only two points influencing the fit here. It was the part of the curve less than 40 mN m^{-1} which was important for the calculation of the critical surface tension (γ_{crit}).

The improved triclopyr uptake observed with Silwet L77 at 0.25% (v/v) in the radiolabeled study cannot be totally explained by the surface tension results. Rate response studies have shown that 0.25 and 0.50% (v/v) rates of Silwet L77 induced significantly greater increase in foliar uptake of radiolabeled triclopyr than 0 and 0.1% treatments.[8] The 0.50% (v/v) rate was not used, as findings suggest that translocation may be reduced at such high rates.[6] In addition, fluorescence studies showed the greatest effect on triclopyr penetration when surfactant rates of 0.25 and 0.50% (v/v) were used. The 0.1% (v/v) rate did not appear to improve the uptake of triclopyr after 60 s, and the improvement after 1 h was considerably less than that obtained with 0.25 and 0.50% (v/v). This was true in spite of the surface tension of all three solutions (0.1, 0.25, and 0.50%, v/v) being essentially the same (Figure 1). This highlights how the interaction of the herbicide-surfactant combination with the plant surface must be of considerable importance.

Retention and penetration of triclopyr amine by the wax was higher then Silwet L77 was present in the formulation. This may be a result of the greater wax area covered by a droplet of herbicide solution when Silwet L77 is present. However, a greater droplet spread due to Silwet L77 was unlikely to be the complete cause of the significant enhancement in overall triclopyr uptake. First, net uptake values (i.e., when ^{14}C-triclopyr in the wax is

removed from the gross uptake) were still significantly greater when Silwet L77 was present than when it was not. This is a reflection of the wax uptake never exceeding 5.0% of the applied triclopyr. As a result, the removal of wax uptake from gross uptake did not significantly change the net uptake trends from those of gross uptake. Second, if the greater droplet spread was the reason for improved uptake with surfactant, the fluorescent micrographs would have demonstrated similar degrees of herbicide absorption within the droplet regions for both control and Silwet treatments. As this was not observed, Silwet L77 must enhance triclopyr amine uptake by some means other than providing a greater contact area for herbicide droplets.

The uptake promotion due to Silwet L77 was very rapid and occurred within 20 min of application (Figure 6). Field and Bishop[12] found the first 3- to 5-h period to be critical in the enhancement of glyphosate uptake by Silwet L77. After periods of 12 to 24 h, glyphosate uptake with Silwet L77 was not significantly different from the control. Uptake studies of triclopyr amine with or without Silwet L77 over periods up to 24 h also indicated that the first 4-h period was the most important in terms of the rapidity and magnitude of Silwet L77's action.[8] A rapid effect on uptake was further demonstrated by fluorescence microscopy studies. When Silwet L77 was present at optimal rates (either 0.25 or 0.5%, v/v), substantial herbicide solution had penetrated the foliar surface just 60 s after application, whereas control and 0.1% Silwet L77 solutions had not (Figures 3 to 5, Table 1).

Triclopyr amine uptake appeared to follow two quite different phases (Figure 6). During the first 45 min, control solutions showed very low rates of uptake, with gross uptake not exceeding 3.0% of the applied triclopyr. The Silwet L77 (0.25%, v/v) treatment entered relatively quickly during this same initial phase, with 6.0 and 6.4% uptake after 20 and 45 min, respectively. A second phase of uptake occurred after 45 min in which the rates of uptake for control and Silwet L77 treatments were similar. The difference between the two lines was constant and was mainly due to the margin established by the first 20 min. It is proposed that the two phases reflect different pathways or means by which triclopyr entered the leaf. The early phase, taking place in the first hour, may be the period in which instantaneous stomatal infiltration occurred, provided the solution's physical characteristics were suitable. Schonherr and Bukovac[18] stated that to achieve this stomatal infiltration, the surface tension of the herbicide solution must be at or below the critical surface tension for that surface. Since the adaxial leaf surface of field bean has a critical surface tension of 19.5 to 22.9 mN m^{-1} (Figure 2), the triclopyr amine solution with 0.25% (v/v) Silwet L77 satisfied this requirement to achieve stomatal entry (Figure 1). Hence, the difference in uptake between the control and Silwet L77 treatment, which was initiated in the first 20 min of uptake, can be attributed to the mass flow and rapid entry of triclopyr via stomata. Fluorescence studies further supported this model, as triclopyr solutions with suboptimal rates of Silwet L77 (0 and 0.1%, v/v) did not significantly penetrate the leaf surface after 60 s or 60 min. In contrast, 0.25 and 0.50% (v/v) Silwet L77 solutions displayed considerably more penetration at both times. Moreover, penetration in these cases was closely associated with stomatal pore regions. Although the 0.1% Silwet L77 treatment should have promoted stomatal infiltration owing to a sufficiently low surface tension (Figure 1), the degree to which this can occur does not equal that of the two higher rates. Thus, surface tension may not be the single determinant in the improvement of triclopyr uptake, due to the addition of the organosilicone surfactant Silwet L77.

Toward the end of the first phase (between 20 and 45 min), the triclopyr uptake rate tended to decrease. This response was particularly noticeable with Silwet L77. It may reflect a saturation of the stomata, more particularly the occlusion of substomatal cavities. The inital rapid penetration rate could not be sustained because the necessary physical gradients could not be maintained as the pore regions approached a satiation level. In the case of the control treatment, any dust, trichomes, or weakened cuticle sites would allow the initial

rapid herbicide entry. Saturation of these sites may be the cause of the apparently small reduction in the control uptake rate between 20 and 45 min.

The second phase uptake pathway (after 60 min) appeared similar for both treatments, since the slope of the curves were the same. At this stage of herbicide uptake (i.e., after 45 min), the process may be one of herbicide diffusion across the cuticle, in which the organosilicone does not seem to play an important role. The rate of herbicide flux through the leaf was similar for both treatments and was equivalent to the flux in phase 1 with the control treatment. This may be a result of the triclopyr solutions requiring time for sufficient attrition of the surface layers and/or of major cellular damage in the epidermal layer. Both changes would allow more rapid movement of the herbicide in phase 2.

Thus, the proposed model of organosilicone-enhanced triclopyr uptake is biphasic. The main effect of the Silwet L77 surfactant was rapid promotion of stomatal penetration into the leaf, occurring within the first 20 min. There was apparent saturation of the substomatal cavities toward the end of the first uptake phase (between 20 and 45 min), reducing the rate of herbicide flux into the leaf. During the early part of phase 1, the significant difference between the control and Silwet L77 treatments was established. Herbicide fluxes in phase 2 were similar for both treatments, which probably reflects a predominance of simple diffusion by both solutions through a partially perturbed leaf surface. Additional research with other organosilicone surfactants and triclopyr suggests that their mode of action may be similar to that of Silwet L77.

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Chapter 7

INFLUENCE OF AN ETHOPROPOXYLATED FATTY AMINE ON THE PENETRATION OF GLYPHOSATE ACROSS ISOLATED TOMATO FRUIT CUTICLES

Simone Santier and André Chamel

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I. INTRODUCTION

Surfactants are widely used to enhance the performance of foliar applied chemicals, but their effects on foliar uptake are not well understood. Various effects are possible: on spray retention, depending on the wettability of plant surfaces; on penetration, by increasing the area of contact with the leaf; by acting as a humectant, keeping the spray droplets moist for a long time; by improving stomatal penetration; by lowering the surface tension of the spray solution; by facilitating movement along cell walls after entry into the foliage; and by lowering interfacial tensions. Surfactants could also influence cuticular penetration by acting as co-solvents or solubilizing agents, or by affecting permeability. To elucidate the effects of surfactants on penetration across the cuticle, the initial and primary barrier to foliar uptake, we investigated this question *in vitro*, using isolated cuticles. In this chapter, we report results concerning the effects of an ethopropoxylated fatty amine (Armoblen 557®) on the penetration of ^{14}C-glyphosate, *N*(phosphonomethyl)glycine, across isolated tomato (*Lycopersicon esculentum* Mill. cv. Marmande) fruit cuticles used as a model of the plant cuticle.

II. MATERIALS AND METHODS

A. PREPARATION OF ISOLATED CUTICLES

Cuticular discs (diameter: 1 cm) were isolated from mature tomato fruit at 35°C using a mixture of 2% pectinase and 0.2% cellulase (Sigma) at pH 3.8.[2] The cuticles were washed several times with deionized water after their separation, then air dried and stored at room temperature. The efficiency of the separation method was checked by observing the internal surface of the isolated cuticles by scanning electron microscopy (Figure 1).

B. EXPERIMENTAL CONDITIONS

The experiments were carried out in an air-conditioned room at 20°C, at either low or high relative humidity, according to the diagram given in Figure 2. In the experiments carried out under high-humidity conditions, the cuticles were set out in an impervious box containing a water reserve.

C. MEASUREMENT OF CUTICULAR PENETRATION

The ^{14}C-glyphosate as the isopropylamine salt in aqueous solution (10^{-4} *M*) was applied, with and without the surfactant, as a droplet (4 μl) on the external surface of a cuticular disc placed on an agar block acting as the receiver. Each receiver block was set at the top of a cut syringe (Figure 2), and thus easily recovered using the piston. This device was used to maintain constant hydration of the agar during the penetration. The cuticles were removed and set on new agar blocks several times after the initial deposit. For the experiments under high-humidity conditions, the impervious box was opened and closed rapidly at each change of receiver block. Rapid decrease of the relative humidity inside resulted nevertheless, which was followed by a slower increase until the maximum value was regained. In this case, therefore, the experimental values obtained for the cuticular uptake greatly depended on the frequency of this opening and closing process. At the end of each experiment, the treated surface of the cuticles was washed and the radioactivity of the washings, cuticular discs, and agar blocks determined separately.

D. CHEMICAL

The ethopropoxylated fatty amine (Armoblen 557) was used because this type of surfactant is recommended for foliar sprays of glyphosate.[7]

FIGURE 1. Internal surface of the isolated tomato fruit cuticle.

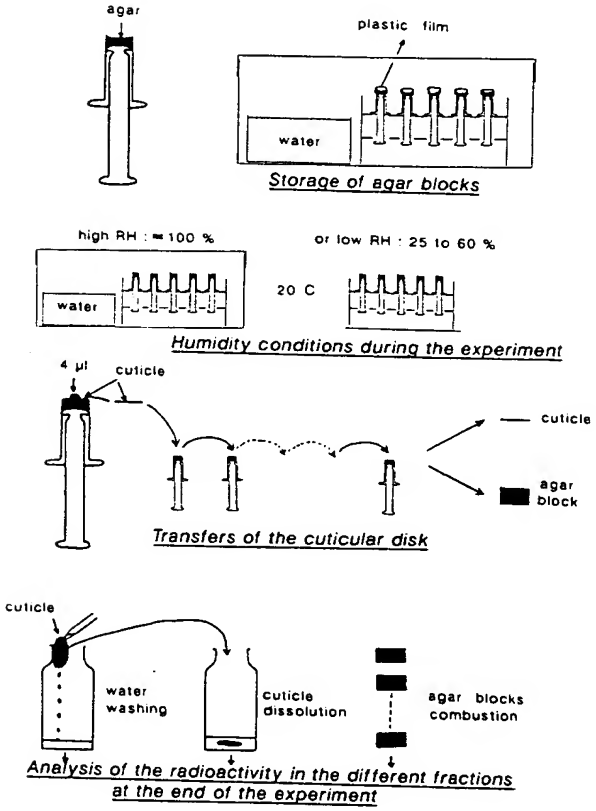

FIGURE 2. Procedure used to study cuticular penetration.

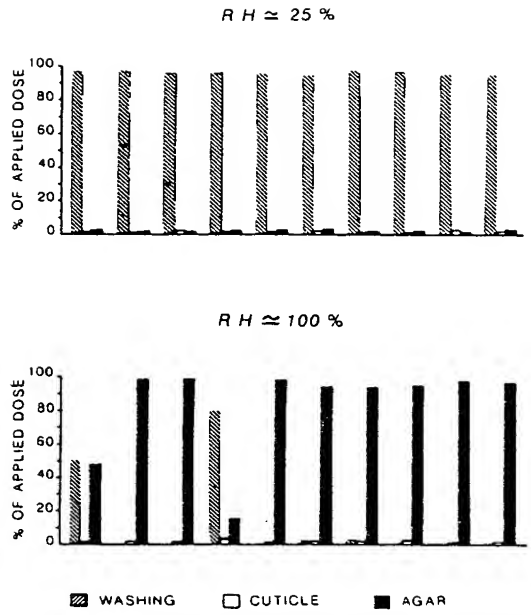

FIGURE 3. Effect of the relative humidity on the cuticular penetration of ^{14}C-glyphosate through isolated tomato fruit cuticles. The results obtained with ten cuticular discs are reported for each set of humidity conditions. Duration of the experiment, 72 h.

III. RESULTS

A. EFFECT OF RELATIVE HUMIDITY

The penetration of glyphosate across the cuticle greatly depended on the relative humidity and was always considerably greater at high rather than low humidity (Figures 3 and 4). The results in Figure 4 also show that a change from low to high or, conversely, from high to low humidity 24 h after the herbicide deposit, when the droplets were no longer visible, resulted in a drastic change in uptake. Kinetic measurements have allowed discrimination of the penetration before and after the evaporation of the droplet. They have revealed that in most cases of high humidity (Figure 5), diffusion in the receiver block was still very limited at the end of the droplet evaporation, occurring indeed mainly after this time. In the experiment reported in Figure 5, the final value of the penetration obtained at 72 h is lower than that in Figure 3. This difference is due to the reasons mentioned in Section II.

B. EFFECT OF SURFACTANT

The effect of the surfactant was investigated under low- and high-humidity conditions.

At high humidity, there was no significant effect of the surfactant on total penetration, but the uptake without surfactant was already very important (Table 1). However, an effect of the surfactant during evaporation of the droplet was noted in kinetic experiments. The values of the uptake at the end of evaporation reached 1.9 and 12.7% of the applied dose for the control and 0.5% surfactant, respectively. Evaporation was two to three times faster with the surfactant under these high-humidity conditions.

Under low-humidity conditions, the glyphosate without surfactant diffused weakly across the cuticle. This diffusion was increased by addition of the surfactant at three concentrations: 0.1, 0.5, and 2.5% (Table 1). Kinetic measurements revealed that the surfactant increased

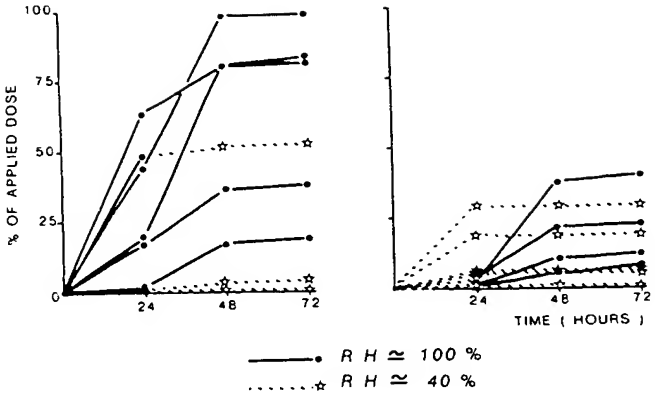

FIGURE 4. Penetration of ^{14}C-glyphosate through isolated tomato fruit cuticles under different conditions of humidity. Each curve corresponds to a given cuticular disc. The receiver blocks were renewed at 24 and 48 h.

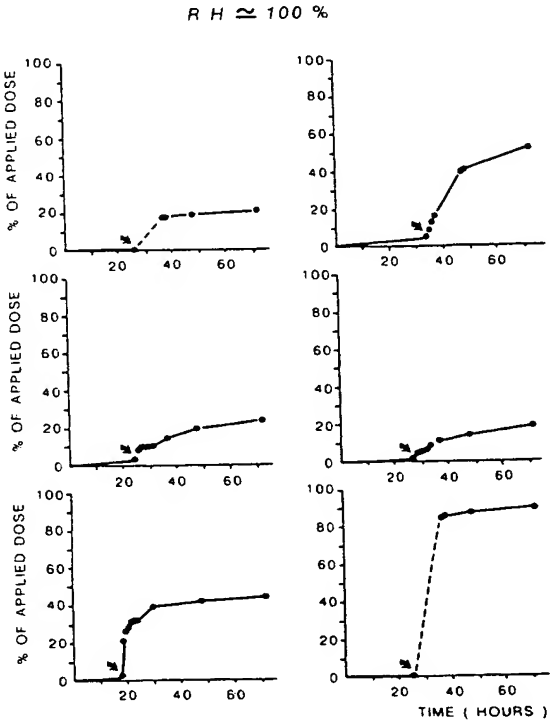

FIGURE 5. Penetration of ^{14}C-glyphosate through six tomato fruit cuticular discs under high humidity conditions. The arrow indicates the end of droplet evaporation. Each black dot corresponds to a change of receiver block. A dashed line was plotted when there was no renewal of the receiver block during the first hours following droplet evaporation.

the duration of penetration for 2 to 3 d after the disappearance of the droplet (Figures 6 and 7). It was also noted that the penetration rate of glyphosate during droplet drying was higher with the surfactant.

TABLE 1
Effect of Armoblen 557® on the Penetration of ^{14}C-Glyphosate Through Isolated Cuticles

Relative humidity (%)	Surfactant conc (%)	Percent of applied dose[a]					
		Surface 72 h	Cuticle 72 h	Agar 0-24 h	Agar 24-48 h	Agar 48-72 h	Total
55	0	93.4	1.4	5	0.2	0	5.2 b
	0.1	63.4	1.7	34.4	0.4	0.14	34.9 c
	0.5	50.3	2.6	43.2	3.4	0.48	47 c
	2.5	67.7	2.8	14.3	10.2	5.06	29.5 c
100	0	12.9	9.7	65.9	6	5.5	77.4 d
	0.1	2.9	0.8	81.3	12.1	2.9	96.3 d
	0.5	13.8	1	72.7	8.9	3.9	85.3 d
	2.5	14	0.6	79.8	3.3	2.3	85.4 d

[a] Numbers are means of eight replications and are significantly different ($p < 0.05$) when followed by different letters.

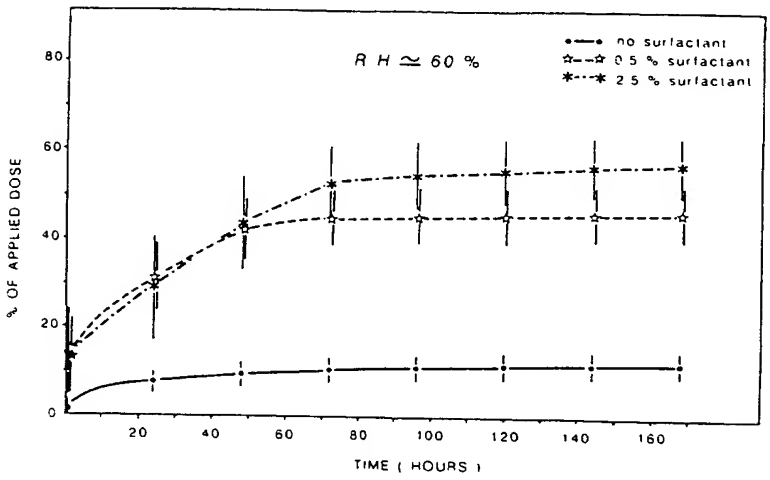

FIGURE 6. Effect of Armoblen 557 on the penetration of ^{14}C-glyphosate through isolated tomato fruit cuticles under low-humidity conditions. There were 7 to 16 replicates for each value. Confidence interval, $p = 0.05$.

IV. DISCUSSION

The surfactant Armoblen 557 increases the penetration of glyphosate through isolated tomato fruit cuticles under low-humidity conditions. It appears to extend the duration of penetration considerably after the apparent disappearance of the droplet. The effect of the surfactant may be partly explained by the increase in contact area between the herbicide solution and cuticular surface. The values of this wetted area were approximately 3, 11, 18, and 28 mm^2 with solutions containing 0, 0.1, 0.5, and 2.5% surfactant, respectively. The values of the uptake calculated per unit area, at the end of droplet evaporation, were approximately the same with and without surfactant for both sets of humidity conditions (mean values of the uptake in percent per square millimeter: 0.61 and 0.72, 0.52 and 0.75,

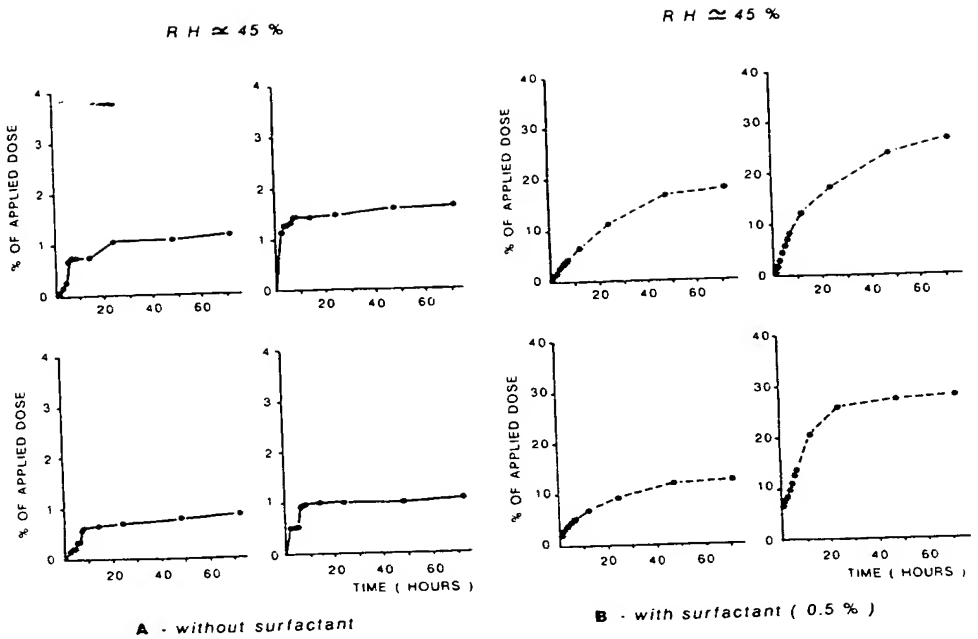

FIGURE 7. Comparison of the penetration kinetics of ^{14}C-glyphosate, with and without Armoblen 557, through isolated tomato fruit cuticles under low-humidity conditions. Examples obtained with four cuticular discs are reported for each set of conditions: (A) without surfactant; (B) with surfactant. The first black dot on each curve corresponds to the end of droplet evaporation. The Y-scales in A and B differ by a factor of ten.

0.12 and 0.11, all for 0.0 and 0.5% surfactant, respectively, at 100, 60, and 40% relative humidity). The increase in the droplet/cuticle contact area, with the surfactant, leads to a decrease in the volume of liquid per unit area and consequently, of the concentration gradient for the herbicide diffusion. The apparent duration of the droplet evaporation was not clearly affected by the surfactant under low-humidity conditions. After droplet evaporation, there was no clear effect of surfactant on the uptake calculated per unit area. The question of the homogeneity of the distribution of the glyphosate on the deposit area after droplet drying was not resolved, making it impossible to consider the influence of the concentration gradient during this step of the uptake. The surfactant at first allowed diffusion of the herbicide through a greater cuticular area, from a lower volume of liquid and a lower concentration gradient by unit of surface; then, after the apparent droplet evaporation, it would maintain the glyphosate, a hydrosoluble herbicide, in the form of a concentrated aqueous film on the cuticular surface. The surfactant may also have a positive effect on the mobility of the herbicide through the cuticle, e.g., by reducing solute-solute intermolecular interactions. This question could be clarified by measurements of cuticular permeability and the partition coefficient, as previously described.[1] In connection with this, the effect of the surfactant on the cuticular structure should be investigated. The effect of humidity on the foliar uptake of glyphosate, already observed in experimentation on whole plants,[3-6] was confirmed by this study using an *in vitro* model.

V. CONCLUSIONS

The experimental model presented was successfully used to study cuticular penetration following localized applications of the herbicide solution.

The penetration of glyphosate across isolated tomato fruit cuticles depends greatly on the relative humidity. It is considerably greater under high- than under low-humidity conditions. The surfactant Armoblen 557 (0.1, 0.5, and 2.5%) also facilitates penetration. Its main effect is attributed to the prolongation of favorable conditions on the cuticular surface, allowing the diffusion of glyphosate through a greater area to be continued after the apparent disappearance of the herbicide droplet.

Recent results reveal that the penetration of glyphosate across cuticles isolated from two other plant species is considerably lower than in the case of tomato fruit cuticles. At this stage in the investigation, the reported effects must be confirmed with other cuticular types, and it must be considered whether similar results can be obtained with other surfactants.

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Chapter 8

INFLUENCE OF THE TYPE AND CONCENTRATION OF SURFACTANT ON GLYPHOSATE ABSORPTION; RELEVANCE OF DROP SPREADING AND DRYING TIME

Hans de Ruiter, Esther Meinen, and Monique A. M. Verbeek

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ABSTRACT

The addition of different concentrations (0.005 to 5.0%) of three cationic polyoxyethylene fatty amine surfactants and a nonionic polyoxyethylene nonylphenol surfactant markedly influenced the foliar uptake of glyphosate [*N*-(phosphonomethyl) glycine], applied to winter wheat (*Triticum aestivum* L.) seedlings. At a concentration of 0.5% (w/v), the surfactant Ethomeen®* T/25 (polyoxyethylene [15] tallow amine) gave the highest absorption rate compared with the other surfactants at 0.5%.

High absorption rates of glyphosate were observed at a concentration of 5.0%. Alone, Ethomeen HT/60 (polyoxyethylene [50] hydrogenated tallow amine) reduced the absorption by a factor of five at high concentrations (2.5 and 5.0%).

The spreading of the drops and their drying time were influenced by surfactant type and concentration. The results indicated that increased drop spreading and a shorter drying time may reduce the absorption of glyphosate. This reduction was observed with Renex®** 688 (polyoxyethylene [8] nolyphenol) and Ethomeen C/12 (polyoxyethylene [2] coco amine) at a concentration of 0.05%, but was not observed at the other concentrations.

I. INTRODUCTION

The phytotoxicity of glyphosate can be influenced by the type and concentration of the added surfactant.[5,9,14] It has been demonstrated that the type of surfactant influences the foliar absorption of this herbicide.[5,6,8,13] For glyphosate and other herbicides, it is not clear to what extent the surfactant concentration influences absorption. Therefore, in this study, three cationic ethoxylated fatty amine surfactants (Ethomeen C/12, Ethomeen T/25, and Ethomeen HT/60) and a nonionic ethoxylated nonylphenol surfactant (Renex 688) were applied at different concentrations in foliar uptake experiments.

The many factors invoked to explain the influence of surfactants on the foliar absorption include droplet spreading, drying times of droplets, herbicide-surfactant interactions, hygroscopicity of surfactants, permeability of the cuticle, and permeability of the cell membrane.[7] The experimental corroboration for these suggestions is slow in coming because of the complexity of the absorption process.

There is some evidence that the uptake of glyphosate during droplet drying is many times faster than from dry deposits.[12] In this study, the drying times were visually determined to see if there was a correlation between absorption and drying time.

II. MATERIALS AND METHODS

A. PLANT MATERIAL

Winter wheat (cv. Arminda) was grown in 9-cm-diameter plastic pots (three plants per pot) filled with a mixture of sand and humic potting soil (1:2). The pots were subirrigated with half-strength Steiner's nutrient solution.[10] The plants were grown in a growth chamber under the following conditions: 15 h light, 18/12°C (day/night), and 70% relative humidity. Light was provided by high-pressure sodium lamps (Philips® 400 W SON/T) and fluorescent lamps (Philips TLD 36 W color 54) to give 80 W/m² at leaf level.

B. SURFACTANTS

The three cationic fatty amine surfactants selected for this study were Ethomeen C/12 (polyoxyethylene [2] coco amine), Ethomeen T/25 (polyoxyethylene [15] tallow amine) and

* Ethomeen and Armoblen are the registered trademarks of AKZO chemicals GmbH, Düren, Germany.
** Renex is the registered trademark of ICI Surfactants, Wilton, England.

FIGURE 1. Generalized structure of the ethoxylated fatty amine surfactants (A) and the ethoxylated nonylphenol surfactants (B).

Armoblen®* NPX containing 50% water and 50% w/v Ethomeen HT/60 (polyoxyethylene [50] hydrogenated tallow amine). The nonionic surfactant was Renex 688 (polyoxyethylene [8] nonylphenol). Each surfactant was a mixture of compounds differing in the length of the alkyl chain and in ethylene oxide (EO) content. Generalized structures of the surfactants are shown in Figure 1.

According to the manufacturers information, in coco amine (Ethomeen C/12), 80% of the alkyl chains range from C12 to C16 in length; in tallow amine (Ethomeen T/25 and Ethomeen HT/60, 95% of the alkyl chains range from C16 to C18 in length, and in hydrogenated tallow amine (Ethomeen HT/60), the unsaturated C–C bond normally present in the alkyl chain is saturated.[1]

C. ABSORPTION OF ^{14}C-GLYPHOSATE

After emergence, the wheat seedlings were thinned to one per pot. Applications were made at the threeleaf stage (14 d after sowing) in the growth chamber. The applications were carried out between 4 and 7 h after commencement of the light period. Methyl-labeled ^{14}C-glyphosate (Amersham, specific activity 2.1 GBq/mmol) was converted to the monoisopropylamine salt by the addition of isopropylamine in a 1:1 molar ratio.

Nonlabeled technical grade glyphosate (monoisopropylamine salt), surfactants, and demineralized H_2O were added to the ^{14}C-glyphosate so that the concentration of glyphosate (labeled plus nonlabeled) was 1.3 m*M*. The surfactants were included on a weight-to-volume basis. The glyphosate solution was applied alone or in combination with surfactant as four 1-µl droplets (0.83 kBq) to a discrete area on the adaxial side of the second leaf. The discrete area was marked with waterproof drawing ink. All applications were made using a Burkard Microapplicator PAX 100 fitted with a 50-µl syringe and needle coated with polytetrafluoroethylene (PTFE). After 24 h, the treated leaf was removed and washed with 5 ml of water. A 0.5-ml aliquot from the wash was added to 5 ml of scintillation liquid (Hydroluma™, Lumac, LSC B.V., Landgraaf, The Netherlands). Radioactivity was quantified using standard liquid scintillation spectrometry techniques. Absorption was defined as ^{14}C not recovered in the wash, and was calculated as the percentage of ^{14}C applied.

Three experiments were carried out, each according to a 4 × 5 triple rectangular lattice design. Replicate treatments were carried out on different days.

D. SPREADING AND DRYING OF DROPS

The conditions of application in relation to the plants were the same as described for the absorption studies. Five drops (1 µl) containing glyphosate and 0, 0.005, 0.05, 0.5, and 5.0% surfactant were applied to one leaf. Spreading was estimated visually 5 min after application and compared with the spreading of the drop containing glyphosate alone. The time required for drying was also assessed visually. Two experiments were carried out, each with three replications.

E. SURFACE TENSION

The surface tension of surfactant-containing solutions was measured according to the du-Noüy ring method.

TABLE 1
Effect of Surfactant Type and Concentration on the Foliar Uptake of ^{14}C-Glyphosate

Surfactant	Absorption of ^{14}C-glyphosate[a] (surfactant conc. %, w/v)				
	0	0.005	0.05	0.5	5.0
No surfactant	38.6[b]				
Ethomeen C/12		31.0[c]	23.6	34.9	76.3
Ethomeen T/25		49.3	47.9	58.5	70.7
Ethomeen HT/60[d]		46.3	31.4	34.5	7.3
Renex 688		45.4	19.9	29.7	63.3

[a] Percent of applied dose.
[b] Absorption values exceeding 54% differ significantly (5% level) from the control treatment.
[c] LSD for all treatments with surfactant is 19.0% (5% level of significance).
[d] The commercial product Armoblen NPX contained 50% Ethomeen HT/60, which means that, in this experiment, Ethomeen HT/60 was applied at 0.0025, 0.025, 0.25, and 2.5% (w/v).

III. RESULTS

A. ABSORPTION OF ^{14}C-GLYPHOSATE

The results of a representative experiment are shown in Table 1. The significance at the 5% level is poor. However, most of the nonsignificant differences between the treatments shown in Table 1 were also observed in two other separate experiments and, therefore, these differences were also considered in the discussion. Ethomeen HT/60 was applied at a half-rate in the experiment shown (Table 1), as the commercial product Armoblen NPX contained 50% water. In two other experiments with double rates of this product, the same trends were observed. None of the surfactants influenced the absorption at a concentration of 0.005%. At 0.05%, Ethomeen C/12 and Renex 688 reduced the absorption. At 0.5%, Ethomeen T/25 gave the highest absorption rate. At this concentration, the absorption rates of Renex 688 and Ethomeen C/12 were enhanced, compared with the rates at 0.05%. At a surfactant concentration of 5%, all surfactants except Ethomeen HT/60 gave a high absorption. Addition of Ethomeen HT/60 at the highest concentration inhibited absorption almost completely.

B. SPREADING AND DRYING TIMES OF DROPS

At a surfactant concentration of 0.005%, spreading and the drying times of the drop were the same as for drops containing glyphosate alone (Table 2). At 0.05%, Ethomeen C/12 and Renex 688 enhanced the spreading of the drops, and this probably caused the greatly reduced drying time. Ethomeen T/25 and Ethomeen HT/60 had little, if any, influence on the spreading of the drops. The drying time was longer for Ethomeen T/25 at 5.0% and Ethomeen HT/60 at 0.5 and 5.0%. The latter surfactant even prevented complete drying during the 24 h after the application. These two surfactants appear to retain a relative large amount of water compared with Ethomeen C/12 and Renex 688.

C. SURFACE TENSION

Measurement of the static surface tension showed that Ethomeen C/12 and Renex 688 reduced the surface tension much more than the other two products. The critical micelle

TABLE 2
Effect of Surfactant Type and Concentration on the Drying Times and Spreading of Drops

Surfactant	Drying time (min) and spreading (surfactant conc. %, w/v)				
	0	0.005	0.05	0.5	5.0
No surfactant	45.6 (3.8)				
No surfactant	—				
Ethomeen C/12		45.1 (4.9)	16.4 (1.6)	7.7 (1.8)	5.2 (1.3)
Ethomeen C/12		—	+	+ +	+ +
Ethomeen T/25		45.9 (4.8)	45.2 (5.4)	44.0 (5.1)	± 180[a]
Ethomeen T/25		—	±	±	±
Ethomeen HT/60		48.7 (4.1)	50.2 (2.9)	[b]	[b]
Ethomeen HT/60		—	—	—	—
Renex 688		42.7 (3.9)	20.9 (2.5)	19.4 (3.7)	18.6 (3.2)
Renex 688		—	+	+	+

Note: SD (n = 6) in parentheses; — spreads like water; ±, some spreading; +, spreads more than water; + +, spreads much more than water.

[a] Exact drying time difficult to assess visually.
[b] The drops were still present 24 h after application.

TABLE 3
Surface Tension-Reducing Ability and HLB Values of the Surfactants

Trade name	Chemical description	Critical micelle concentration[a] (cmc) (%, w/v)	γ at cmc (mN m^{-1})	HLB[b]
Ethomeen C/12	Ethoxylated (2) coco amine	0.004	28.4	10.2
Ethomeen T/25	Ethoxylated (15) tallow amine	0.01	41.9	19.3
Ethomeen HT/60	Ethoxylated (50) h.[c] tallow amine	0.1	48.1	19.7
Renex 688	Ethoxylated (8) nonylphenol	0.01	31.3	12.3

[a] Measured at 25°C.
[b] Hydrophile-lipophile balance (HLB) according to the manufacturer's information.[1,2]
[c] Hydrogenated.

concentration was 0.004% for Ethomeen C/12, 0.01% for Ethomeen T/25 and Renex 688, and 0.1% for Ethomeen HT/60 (Table 3).

IV. DISCUSSION

A. GLYPHOSATE ABSORPTION

1. Surfactant Type and Concentration

It has been demonstrated that glyphosate absorption depends on the type of surfactant and the surfactant concentration. At a concentration of 0.5%, the surfactant Ethomeen T/25 containing 15 mol of EO enhanced the absorption more than Ethomeen C/12 containing 2 mol of EO and Ethomeen HT/60 containing 50 mol of EO. If it is assumed that the difference between the hydrophobic moiety of Ethomeen C/12 and the hydrophobic moiety of the other two alkyl amine surfactants (mentioned in Section II) has a minor influence, this result agrees with published data for the foliar uptake of 2D-glucose[11] and paraquat[4]

using other types of surfactants. In those experiments, maximum absorption was also observed with surfactants containing an intermediate number of EO groups.

Under our experimental conditions, the nonionic surfactant Renex 688 had much less ability to enhance the glyphosate absorption than the cationic surfactant Ethomeen T/25 (Table 1).

A similar result was found with a study on glyphosate absorption in field bindweed (*Convolvulus arvensis* L.).[8] The cationic surfactant MON 0818 (polyoxyethylene tallow amine) enhanced the absorption, whereas the nonionic surfactant Tween 20 (polyoxyethylene [20] sorbitan monolaurate) had no significant influence.

An evaluation of the ability of surfactants to enhance glyphosate toxicity to common milkweed (*Asclepias syriaca* L.) and hemp dogbane (*Apocynum cannabinum* L.) revealed that cationic surfactants (ethoxylated amine type) were generally more effective than the nonionic surfactants.[14]

To what extent the differences mentioned are due to the charge of the surfactant molecules or to other chemical characteristics remains to be elucidated by developing insight at the molecular level.

2. Drop Spreading and Drying Time

The spreading of the drops on the surface of wheat leaves (which are difficult to wet) was influenced most by Ethomeen C/12 and Renex 688 (Table 2). The property of these products to reduce the surface tension of the solution much more than the other two surfactants (Table 3) probably accounts for this result. Enhancement of the concentration of Ethomeen C/12 increased spreading and shortened the drop drying time, whereas the surface tension of the solutions containing 0.05, 0.5, and 5.0% Ethomeen C/12 and glyphosate (1.3 m*M*) was the same.The spreading of a drop is also influenced by the surface tension of the solid-liquid interface and the surface tension of the solid.[3] Reduction of the surface tension of the solid-liquid interface at concentrations of Ethomeen C/12 higher than the critical micelle concentrations (Table 3) may possibly account for the increased spreading. However, other factors may also be relevant, as the dynamics of drop spreading on a solid surface are not completely understood. At a surfactant concentration of 0.005%, the spreading and drying time of the drop remain unchanged. The influence of the surfactants on the absorption of ^{14}C-glyphosate at this concentration was similar. At a concentration of 0.05%, the increased droplet spreading and shorter drying time observed with Ethomeen C/12 and Renex 688 may explain the reduced absorption of glyphosate if it is assumed that a shorter drying time leads to more rapid immobilization of glyphosate on the leaf surface. At the higher surfactant concentrations (0.5 and 5.0%), absorption with Renex 688 and Ethomeen C/12 was enhanced, notwithstanding the further increased spreading and shorter drying time observed with Ethomeen C/12. This effect may result from the influence of the surfactant on cuticle permeability, the influence on the underlying tissue, or the ability of the surfactants to retain water in the film of surfactant that covers the leaf surface after droplet drying. These factors may also lead to enhanced absorption in the presence of Ethomeen T/25, because this surfactant has little, if any, effect on droplet spreading or drying time (except for Ethomeen T/25 at 5.0%).

Ethomeen HT/60 did not influence the drop spreading and drying time at concentrations of 0.0025 and 0.025% (Table 2). At concentrations higher than 0.025%, the drop did not dry under our experimental conditions. Glyphosate absorption at 0.25% has the same level as measured without surfactant, but at 2.5%, the absorption was strongly inhibited. The results of this study do not indicate what mechanism causes the hindered glyphosate diffusion into the plant tissue when this long EO-chain surfactant is applied at a high concentration.

At present, there is little information on the relationship between foliar absorption and drying times.[12,15] This study indicates that spreading and drying time can be relevant uptake-determining factors. However, to verify the tentative conclusions of this study, more data are required on absorption soon after droplet application and on drying times at different humidities. The influence of the EO content on the retention of water in the film of surfactant covering the leaf surface after visible drying needs more attention, as this retention may influence the absorption of water-soluble compounds. The events in the drying deposits are not the only factors determining the absorption of a compound. Nevertheless, we believe that more insight will lead to generalizations useful for the development of improved formulations.

ACKNOWLEDGMENTS

The authors acknowledge AKZO Chemie for providing Ethomeen C/12, Ethomeen T/25, and Ethomeen HT/60, ICI Specialties for providing Renex 688, and B. V. Luxan for providing technical grade glyphosate. We also thank Mr. J. C. M. Withagen for the statistical treatment of the data.

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Chapter 9

THE EFFECT OF A RANGE OF NONYLPHENOL SURFACTANTS ON CUTICLE PENETRATION, ABSORPTION, AND TRANSLOCATION OF WATER-SOLUBLE AND NON-WATER-SOLUBLE HERBICIDES

Ralph C. Kirkwood, Heather Knight, Irene McKay, and J. P. Nimal R. Chandrasena

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ABSTRACT

The effect of a range of nonylphenol surfactants containing 4 to 14 mol of ethoxylate (EO 4 to 14) was tested on the uptake and distribution of ^{14}C-asulam {methyl[(4-aminophenyl)sulfonyl]carbamate)} or ^{14}C-diflufenican {(*N*-[2-4-difluorophenyl]-2-[3-trifluoromethylphenoxyl-3-pyridine carboxamide]}. Four 0.5-μl droplets were applied to leaves of bracken-fern (*Pteridium aquilinum* L. Kuhn) and common chickweed (*Stellaria media* L.), the herbicidal dose being equivalent to normal field rates. Results show that irrespective of surfactant, uptake and translocation of asulam (hydrophilic) was greater than diflufenican (lipophilic), cuticle retention of the latter being considerably greater than asulam. The uptake of asulam was generally enhanced by surfactants of EO 4 to 10, optimum EO 8, while that of diflufenican was significantly enhanced only by EO 4. The results are discussed in relation to the criteria determining penetration of the cuticle and subsequent transport within the plant.

I. INTRODUCTION

The surface of plant shoots is covered by a lipoidal cuticle which restricts water loss from the plant and acts as a barrier to the penetration of foliage-applied compounds, particularly those having polar characteristics. The outer wall of the epidermal cells is covered by a "cuticular membrane" composed of polymeric cutins with embedded waxes ("cuticular waxes") and an outer deposit of generally structured waxes ("epicuticular waxes").[4,5] The physicochemical properties of these waxes may be very important in determining the wettability of the leaf surface.

Surfactants may enhance cuticle retention and penetration due to a number of functions, including reduction in surface tension, which reduces the droplet contact angle and enhances spray retention, wetting, and spreading. They also may act as humectants, maintaining the active ingredient (a.i.) in the aqueous phase for a longer period, or may possess solubilizing properties which facilitate partition of the a.i. from the solid to liquid phases. In addition, the surfactant may facilitate permeability of the plasmalemma,[10] and this may increase herbicidal efficacy. There is evidence that certain surfactants may enter the leaf tissues, and the elucidation of the relationship between structure and phytotoxicity has been investigated by Silcox and Holloway[8] and Lownds and Bukovac.[7]

The aim of the present study was to examine the effect of a range of ethylan surfactants having differing numbers of ethylene oxide (EO) groups on the uptake and translocation of hydrophilic (asulam) and lipophilic (diflufenican) herbicides.

The test species used in this study are known to be difficult weeds and to present particular control problems. Common chickweed is regarded as a difficult annual species of arable crop while brackenfern is a notorious weed of upland pastures, spreading by growth of the underground rhizome system. The effect of a range of nonylphenol surfactants on the uptake and translocation of asulam and diflufenican has been investigated; these herbicides were selected on the basis of their very different water/lipid solubilities.

II. MATERIALS AND METHODS

A. PLANT TISSUES

Bracken rhizomes, cut into 15-cm fragments and planted in John Innes II compost mixed with bracken litter, were placed in a greenhouse. Subsequently, uniform fronds were selected and pinnae removed from the base of mature fronds. The stem of each pinna was inserted

TABLE 1
Nonylphenol Surfactants EO 4 to 14

EO	Compound name	Manufacturer
4	Ethylan 44	Lankro Chemicals
5.5	Ethylan 55	Lankro Chemicals
6.5	Ethylan 77	Lankro Chemicals
8	Ethylan TU	Lankro Chemicals
10	Synperonic NP 10	ICI
12	Ethylan DP	Lankro Chemicals
14	Lutensol AP 14	BASF

into a vial containing 8 ml of distilled water. A photosynthate "sink" was achieved by covering the apical pinnules with aluminum foil. The explants were placed in a growth cabinet for 24 h prior to herbicide treatments (normally 25°C ± 1°C; [RH], 75% ±5%, 14-h photoperiod).

Chickweed seeds were planted in 7-cm pots in John Innes II compost and grown to the cotyledon state in the greenhouse; no sink was created in this species. The potted plants were placed in the growth cabinet for 24 h prior to treatment.

B. HERBICIDES AND ADJUVANTS

The ^{14}C-asulam (sodium salt) used was ring labeled with a specific activity of 10.1 mCi mmol^{-1} (44 μCi mg^{-1}); ^{14}C-diflufenican was pyridine-ring labeled with a specific activity of 2.0 mCi mmol^{-1} (5.1 μCi mg^{-1}). The relative solubilities of asulam in water and acetone were 4 and 300 g l^{-1}, respectively; the relevant figures for diflufenican were 0.05 ppm and 10%, respectively. A range of nonylphenol surfactants containing 4 to 14 mol of ethoxylate (EO 4–14) were used as adjuvants at a concentration of 0.1% (Table 1).

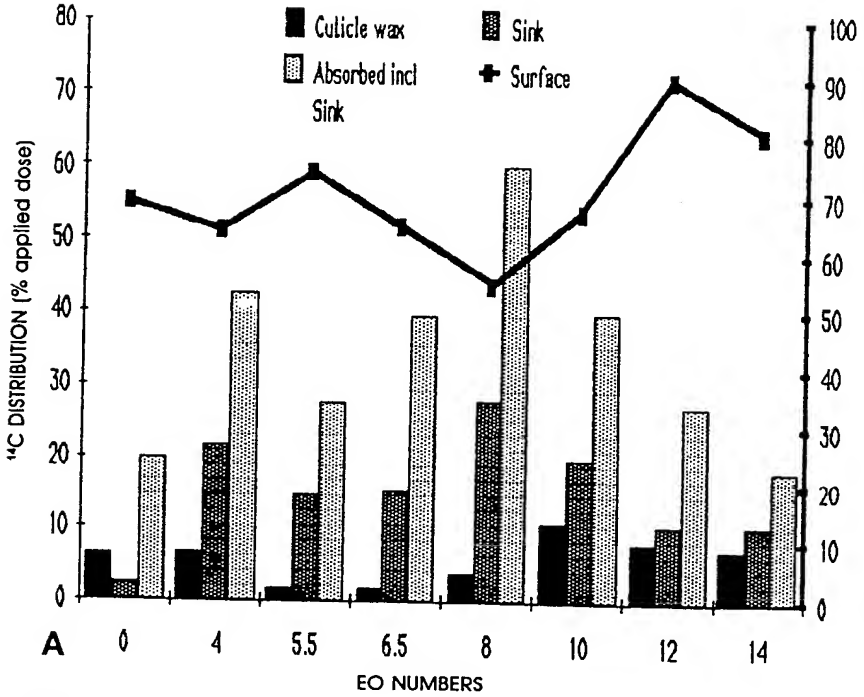
^{14}C-asulam and ^{14}C-diflufenican were applied in solution at the equivalent of field rates (7.5 l/300 l and 200 g/200 l ha^{-1}, respectively). In the case of bracken, eight 0.5-μl droplets of the appropriate herbicide were applied to the basal pair of pinnules (total of 4 μl); an equivalent amount was applied to the cotyledons of common chickweed.

After 48 h, the plants were harvested, and the treated tissue was separated from the untreated and washed in 3 ml of water + acetone (2 min) and 2 ml of chloroform (30 s) to remove ^{14}C-residues associated with the surface or cuticle waxes. The treated and untreated regions were dried at 50°C, weighed, wrapped in filter paper, pelletized, combusted (Packard Tricarb Sample Oxidizer, B306), and the $^{14}CO_2$ formed was trapped in 8 ml of Carbosorb® II and dissolved in 10 ml of Permafluor scintillant for radioassay.

The data obtained were subjected to analysis of variance, and where relevant, Duncan's multiple range test (D test).

III. RESULTS

The effect of the ethylan series on absorption and translocation of ^{14}C-asulam by bracken fern is shown in Figure 1A. Absorption was significantly enhanced by surfactants of EO 4 to 10, EO 8 being optimum; sink accumulation followed a similar pattern. Calculation of the ratios of absorption/surface + wax residues (A/R), sink accumulation/absorption (S/A), and translocation (sink + nonsink)/absorption (T/A) are shown in Figure 1B. The results emphasize the importance of surfactant EO in relation to absorption, effects on translocation being secondary.



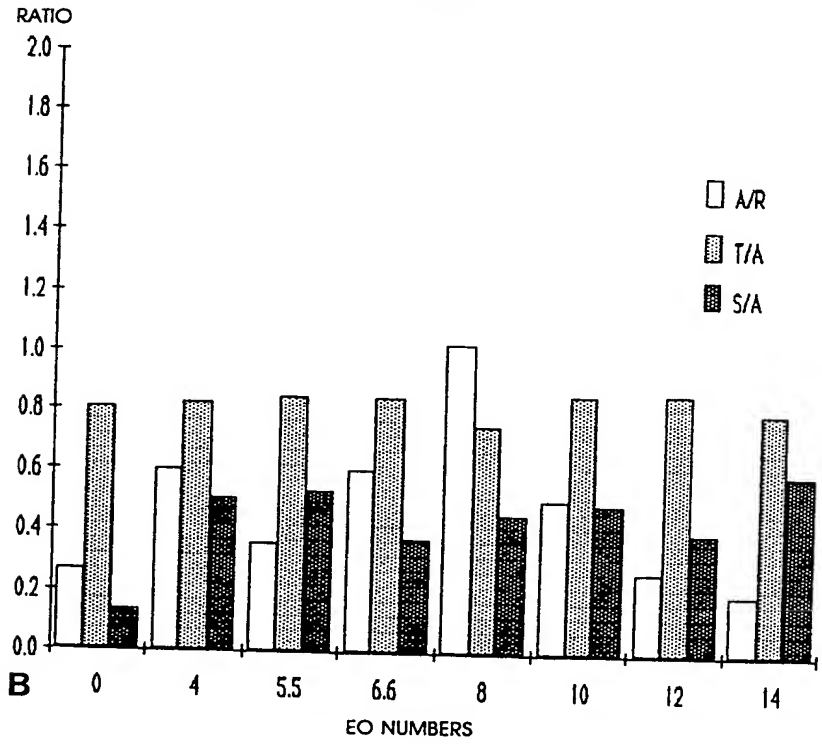


FIGURE 1. The effect of ethylan surfactants (EO 4 to 14) on the distribution of ^{14}C-asulam applied to brackenfern. (A) Percent of applied dose; (B) absorption to surface residues (A/R), translocation to absorption (T/A), and sink accumulation/absorption (S/A) ratios.

In the case of ^{14}C-diflufenican applied to bracken fern, incorporation of ethylan surfactants was beneficial to absorption and translocation only at EO 4 (Figure 2A). As EO increased, a marked rise in surface residues was coincident with a gradual reduction in cuticle wax levels and in uptake/movement. The decline in the ratios of S/A and particularly A/R confirm that only surfactant of low EO has a beneficial effect on the uptake and translocation of this lipophilic herbicide; T/A was unaffected by surfactant EO level (Figure 2B).

The effect of the surfactants on absorption and translocation of ^{14}C-asulam by common chickweed is shown in Figures 3A and B. Absorption, but not translocation, was significantly increased by ethylans of EO 4 to 10, with an optimum of EO 6.5 to 8, surface residues of ^{14}C-asulam being reduced proportionately; the low level of translocation was little affected by EO level. Ethylan surfactants had no significant effect on the uptake and transport of ^{14}C-diflufenican, although cuticle wax retention was significantly reduced at EO 6.5 (Figure 4A). The A/R and T/A ratios indicate that absorption and especially translocation declined at the higher EO numbers (Figure 4B).

IV. DISCUSSION

The results presented here indicate that in brackenfern and to a lesser extent common chickweed, the nonylphenol surfactants tested do selectively stimulate the uptake and translocation of asulam and diflufenican. The uptake of asulam, which is relatively hydrophilic, was enhanced by surfactants of low to medium chain length, particularly around EO 8.0; the absorption of lipophilic diflufenican, however, was increased only by surfactant of EO 4, the most lipophilic of the test adjuvants.

These results tend to confirm the findings of Stevens and Bukovac,[9] who examined the uptake of 2-deoxy-D-glucose, atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and DDT [1,1,1-trichloro-2,2-*bis*(4-chlorophenyl) ethane], which have water solubilities of about 50 g l^{-1}, 40 mg l^{-1}, and 17 g l^{-1}, respectively. Uptake of glucose was directly related to the water content of the surfactants at 80% RH, and this content increased with both RH and the log EO content of the surfactant. They believed that the surfactants enhanced the uptake of 2D-glucose by maintaining the chemical in solution on the leaf surface, and found evidence to indicate that phytotoxic adjuvants (low EO) do not necessarily enhance uptake of glucose, unlike DDT or atrazine. These workers showed that uptake of these lipophilic compounds increased with the uptake of the surfactants, being inversely related to their hydrophile-lipophile balance (HLB). They suggested that the increased uptake of atrazine and DDT may have been associated with copenetration of the surfactants. It is noteworthy that the water solubilities of these relatively lipophilic compounds were increased up to eightfold by surfactants, particularly at intermediate HLBs. They concluded that surfactants with short EO chains would be adjuvants of choice to maximize the uptake of compounds of nonpolar active ingredients, while those with long EO chains would be preferable for water-soluble active ingredients.

In the present study, the incorporation of ethylan surfactants having a relatively high number of EO groups (asulam, >10; diflufenican, >5.5) generally was counterproductive, since the surface residues of herbicide increased markedly and the levels of absorption and translocation diminished. The partitioning of ^{14}C-diflufenican into the cuticle waxes was also reduced in the presence of these surfactants, and it may be conjectured that this step reflects an initial rate-limiting phase in the process of cuticle penetration.

Comparison of the efficiency of uptake (percent of applied dose) indicates that for asulam in brackenfern, there was little relationship between uptake and the levels of ^{14}C detected

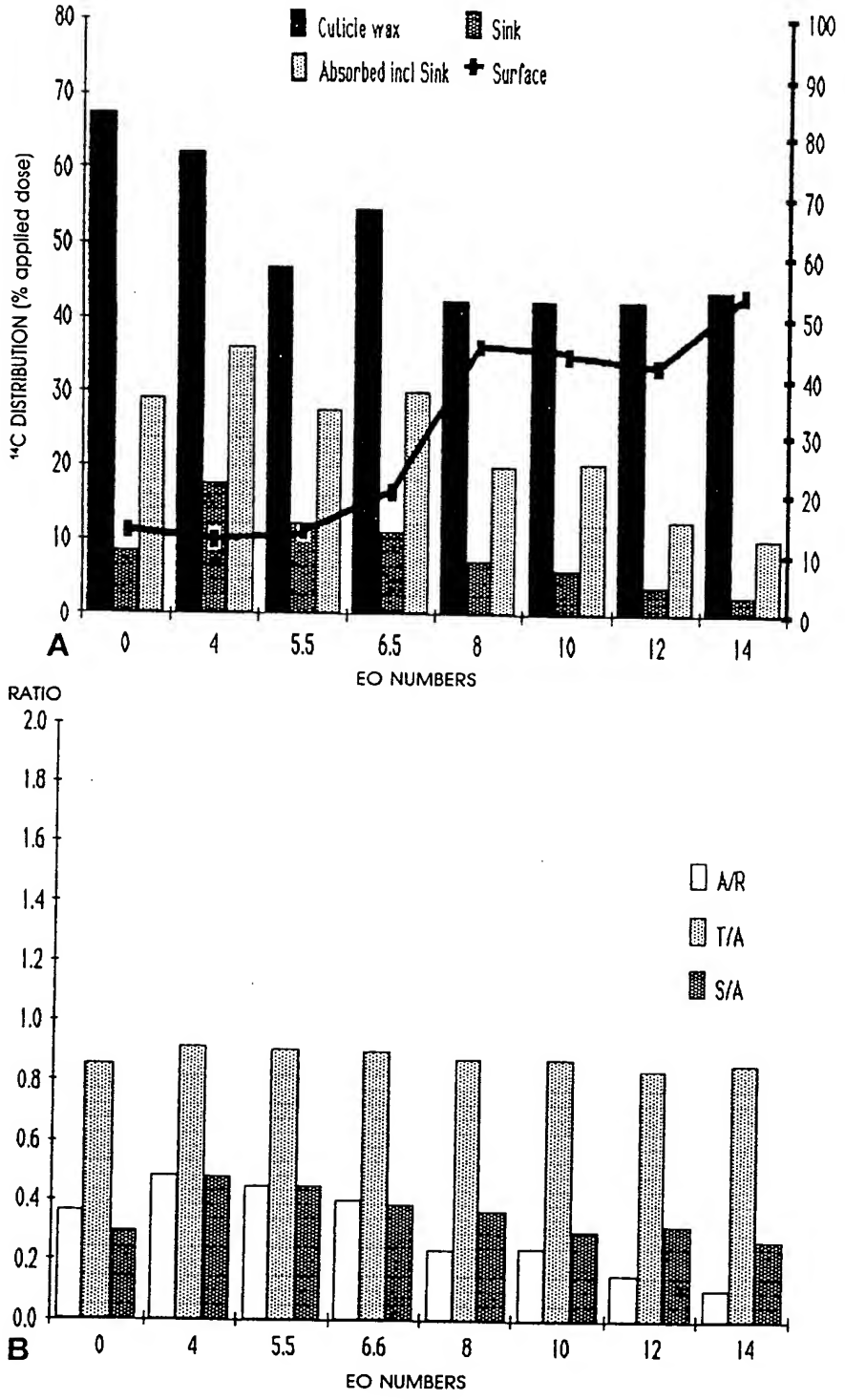


FIGURE 2. The effect of ethylan surfactants (EO 4 to 14) on the distribution of ^{14}C-diflufenican applied to brackenfern. (A) Percent of applied dose; (B) as ratios of A/R, T/A and S/A.

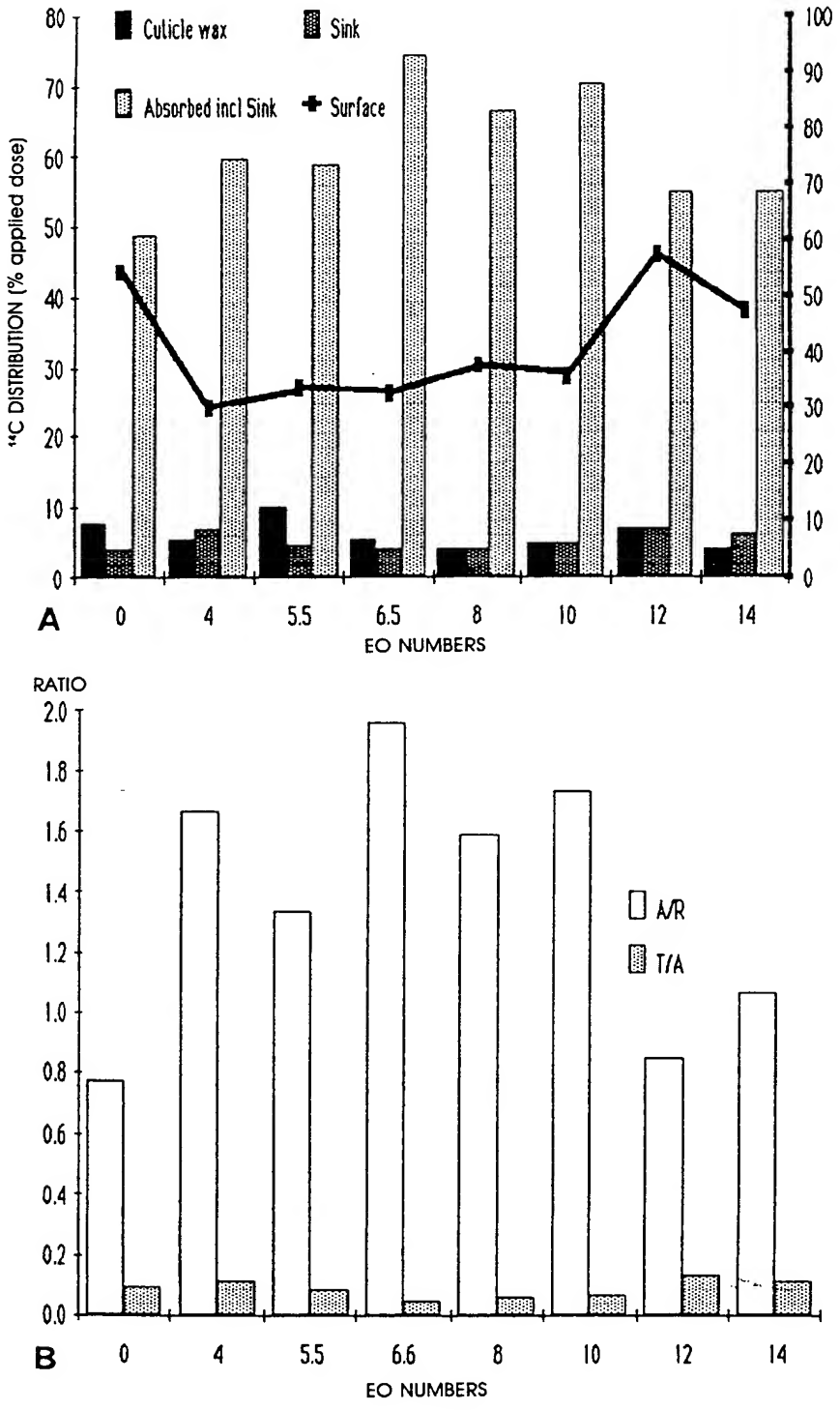


FIGURE 3. The effect of ethylan surfactants (EO 4 to 14) on the distribution of ^{14}C-asulam applied to common chickweed. (A) Percent of applied dose; (B) as ratios of A/R and T/A.

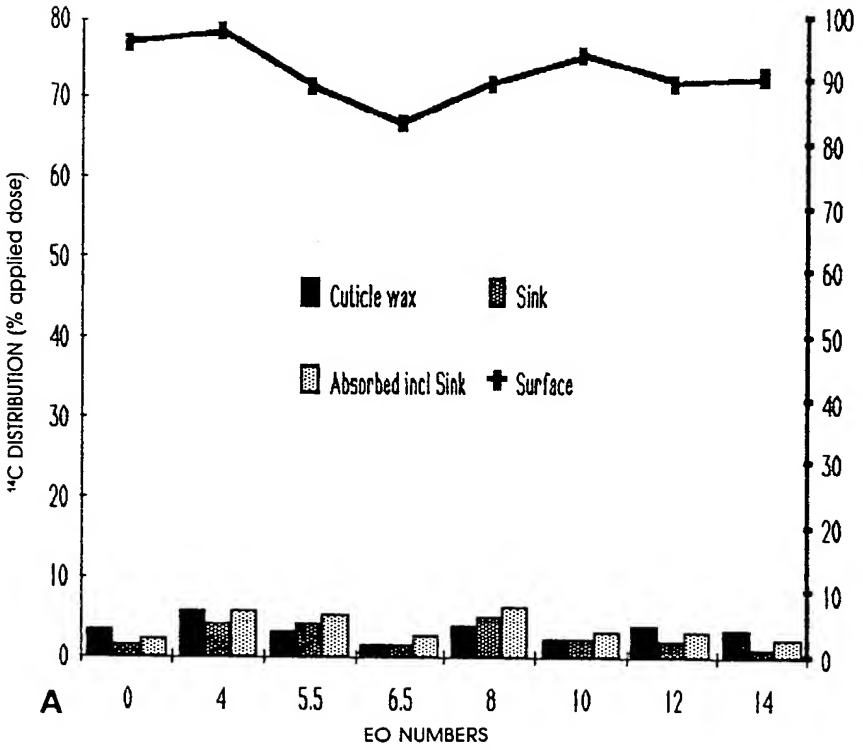


FIGURE 4. The effect of ethylan surfactants (EO 4 to 14) on the distribution of ^{14}C-diflufenican applied to common chickweed. (A) Percent of applied dose; and (B) as ratios of A/R and T/A.

in the waxes. It may be that the flux of transport across the waxes is relatively low and that a relatively more hydrophilic pathway is involved which avoids the waxes. In the case of diflufenican, however, uptake and distribution in the waxes tended to be parallel, with the exception of EO 4. It could be conjectured that partitioning into the waxes may be an initial stage in cuticle penetration and that a lipid route was involved. Surfactant EO levels above 4.0 appeared increasingly to inhibit the efficiency of both processes.

In the case of ^{14}C-asulam, the existence of an optimum of about EO 8.0 may reflect the need for an optimum HLB. An element of lipophilicity presumably enhances penetration of the cuticle membrane and absorption through the plasmalemma, while an appropriate hydrophilicity would help to maintain the chemical in solution on the surface and ensure effective partitioning into the aqueous phase in the inner regions of the cuticle membrane. Asulam is phloem systemic, and previous studies have indicated that the primary effect of nonionic surfactants such as Silwet® L-77 is associated with absorption, while the effects on phloem transport are secondary.[3]

The evidence presented in Figures 1A and 2A indicates that translocation of both compounds mirrors absorption; transport mechanisms per se appear to be largely unaffected. Calculation of the ratios of translocation or sink accumulation to absorption, however, indicates that in brackenfern, the relative sink accumulation of ^{14}C-asulam was increased in the presence of all surfactants. It is known that nonionic surfactants can penetrate the leaf tissues,[8] possibly resulting in enhanced membrane permeability and short-distance transport of phloem-systemic, relatively low phytotoxic compounds, such as asulam.[3]

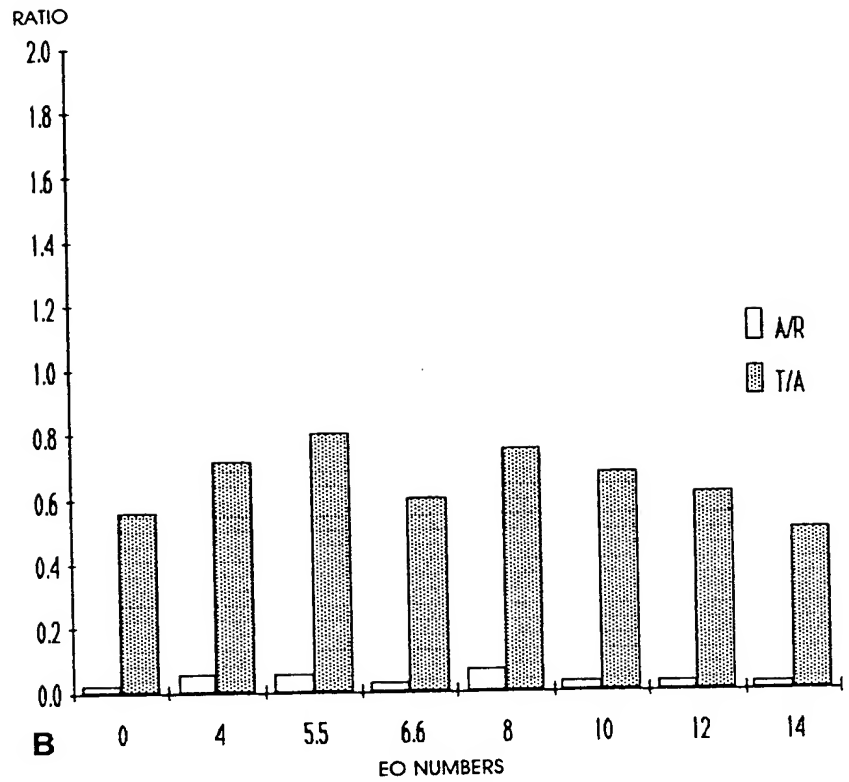


FIGURE 4 (continued).

While a high proportion was present in the cuticle waxes, especially at higher EO levels, the uptake (dpm) of diflufenican was greater than that of asulam. Translocation of the asulam, however, was considerably greater than that of diflufenican, particularly at EO 8, indicating that for effective absorption and phloem translocation, a foliage-applied herbicide requires a suitable HLB. It must be sufficiently lipophilic for cuticle and membrane penetration to occur, and it must be sufficiently hydrophilic to enable partitioning into the aqueous phase in which transport occurs. The importance of HLB has been discussed by Edgington[2] in relation to systemic fungicides. He emphasized that the permeability of nonionized compounds (such as asulam and diflufenican) should be sufficient to enable them to penetrate the phloem, but not so great that loss from the phloem would occur during translocation. Bromilow et al.[1] assessed the retention of a series of chemicals having varying octan-1-ol/H_2O partition coefficients (log K_{ow}) in the phloem and found that compounds with log K_{ow} 0 to 5 had a high retention, while those with values of <1.0 were poorly retained. The results of the present studies are consistent with these findings, since the log K_{ow} for asulam is 0.3, while that for diflufenican is 4.9.

The beneficial effect of these nonionic surfactants lies in improved uptake, with any effects on translocation being secondary. The mechanisms by which cuticle penetration of diflufenican (lipophilic) is enhanced by EO 4 surfactants (lipophilic) is unknown. It may be conjectured, however, that this surfactant enhances partition of this herbicide into, and possibly out of, the cuticular waxes. Retention in the waxes appears to be minimized at EO

4, unlike the situation at higher EO numbers. Enhanced water solubility of diflufenican by surfactants of higher EO number does not appear to be beneficial. In the case of asulam, the role of surfactants of EO 6.5 to 8.0 is uncertain, but they may improve the HLB of asulam, thereby facilitating transcuticle or transmembrane transport. The underlying explanations of these findings will be investigated further.

ACKNOWLEDGMENTS

Appreciation is expressed in the British Council and the Royal Society of Edinburgh for financial support to Professor Chandrasena, enabling this study to be carried out. Grateful thanks are due to Rhone Poulenc for the gift of ^{14}C-asulam and ^{14}C-diflufenican, and to Dr. A. H. Catchpole of Rhone Poulenc for helpful comments. Grateful thanks are expressed to Mrs. Sheila McMillan for preparation of this manuscript.

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Chapter 10

INFLUENCE OF ETHYLAN AND AMMONIUM SULFATE ON GLYPHOSATE PHYTOTOXICITY TO QUACKGRASS (*Elytrigia repens*)

Gilles D. Leroux and Gilles Hamel

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ABSTRACT

In greenhouse experiments, additions of 0.5% (v/v) ethylan or 5.0% (w/v) ammonium sulfate increased the permeability of quackgrass (*Elytrigia repens*) leaf-cell membrane treated with a solution containing 250 mg l^{-1} of technichal-grade glyphosate-isopropylamine salt (MON 0139) [*N*-(phosphonomethyl)glycine] or formulated Roundup® (hereafter, glyphosate). Permeability reached a plateau at different times, depending upon the treatment. Rhizome bud viability was bioassayed using a 0.1% solution of 2,3,5-triphenyl tetrazolium chloride. Mean bud viability was reduced more by glyphosate than MON 0139, regardless of the concentrations (250, 750, and 1250 mg l^{-1}). All treated and untreated plants had increasing bud viability with increasing distance from the treated shoot, but the viability in untreated plants and those treated with 250 mg l^{-1} MON 0139 increased at a greater rate than the ones treated with glyphosate with increasing distance from the treated shoot. Additions of 0.5% (v/v) ethylan or 5.0% (w/v) ammonium sulfate increased by about 25% the phytotoxicity to quackgrass of sprays containing 250 mg l^{-1} glyphosate. Neither adjuvant modified the distribution of glyphosate in rhizomes, compared to glyphosate alone, but rhizome growth subsequent to glyphosate treatment was less with the addition of ammonium sulfate or ethylan than with glyphosate alone.

I. INTRODUCTION

Quackgrass is a persistent perennial grassy weed that infests most crops of humid temperate climate.[18] Effective quackgrass control was made possible with the advent of systemic herbicides able to reach rhizome buds in lethal concentration. Glyphosate, a postemergence, nonselective, systemic herbicide, has been shown to be highly effective against quackgrass,[3,12,14] but high rates are expensive and long-term control may not be obtained with low rates due to inadequate killing of rhizome buds.

Adjuvants include inorganic salts and surfactants that are added to herbicides to improve their efficiencies. The use of surfactants as adjuvants to enhance herbicide activity has been well documented for several herbicides. About 50 years ago, ammonium sulfate (AMS) was used to activate dinitro-*o*-cresol herbicide, DNOC.[4,7] More recently, it was shown that AMS can activate several water-soluble leaf-applied herbicides, including glyphosate.[16,19] Blair[1] has shown that AMS increases the phytotoxicity of glyphosate to quackgrass. In the U.K., the half-dose (0.72 of acid equivalent (a.e.) per hectare) had almost as much effect as the full dose of glyphosate.[8] Occasionally, marked increases in activity were observed when AMS (50 g l^{-1}) was added to the spray solution.[17]

The cationic surfactant Frigate® (fatty amine ethoxylate; hereafter, ethylan) has been found to enhance the activity of the commercial formulation of glyphosate and is recommended for use.[16] Evidence exists that surfactants increase spray retention and subsequent penetration of leaves by herbicides. Surfactants may also solubilize the waxy cuticles of plants, thereby facilitating herbicide entry.

To determine whether leaf cell membrane is altered, inorganic salt leakage from foliar tissues can be measured by incubating them in herbicide solution with adjuvant, in comparison to the herbicide itself and distilled water.[11] Adjuvants applied to plant foliage can change the integrity of the leaf-cell membrane. Increased permeability of the leaf-cell membrane may explain why adjuvants give greater penetration and subsequent translocation of herbicides in plants.

Indirect methods to bioassay the viability of vegetative buds include germination tests in soil,[3,9] agar,[10] and water-saturated substrates.[2] These methods detect only those buds that

have the immediate potential to grow, not those that are dormant.[5] The respiratory activity of cells can be directly evaluated by using triphenyl tetrazolium chloride.[13] This method has the advantage of assessing the viability of both dormant and nondormant quackgrass rhizome buds.[5,15]

The objectives of the study were to (1) compare the influence of formulated Roundup® (glyphosate) to that of technical glyphosate isopropylamine salt (MON 0139), with or without AMS or ethylan, on leaf-cell membrane permeability of quackgrass, (2) compare the effects of increasing rates of glyphosate to those of MON 0139 on quackgrass bud viability, and (3) evaluate the effects of AMS and ethylan on quackgrass rhizome bud viability as affected by increasing rates of glyphosate.

II. MATERIALS AND METHODS

Quackgrass (Laval University clone no. 2) was propagated in the greenhouse (16-h photoperiod; 20 ± 3°C; RH, 45% by planting three-bud rhizome segments in 17-cm pots filled with potting mix consisting of peat moss, decomposed organic soil, sand, vermiculite, and perlite (2:2:2:1:1, v/v/v/v/v). Plants were irrigated twice a day and fertilized weekly with 100 ml of 20-20-20 at 4 g l^{-1}.

A. EXPERIMENT 1

When quackgrass plants reached the six-leaf stage, 15 foliar discs (5-mm diameter) from the second-oldest leaf were placed in a 60-ml test tube with 5 ml of treatment solution. The electrical conductivity of the solution was monitored at various intervals over a 48-h period with a Copenhagen conductivity meter. There were nine treatments: formulated glyphosate and MON 0139 (an isopropylamine salt formulation of glyphosate without wetter), each used at 250 mg a.e. l^{-1}, ethylan at 0.5% (v/v), AMS at 5.0% (w/v), and combinations of glyphosate or MON 0139 with or without ethylan or AMS. Distilled water was used as an untreated control. Variation in conductivity was assumed to result from solute leakage from leaf discs.

B. EXPERIMENT 2

The influence of increasing rates of glyphosate or MON 0139 on bud viability was studied. Plants were propagated as above. The first emerging shoot was identified as the primary stem. The plants were grown for 40 d in the greenhouse. Before treating the plants, the number and position of buds on the primary rhizome were mapped, and the length of the rhizome was measured. The primary aerial stem was dipped for 30 s in herbicide solution containing 0, 250, 750, and 1250 mg a.e. l^{-1} of either glyphosate or MON 0139. The plants were allowed to dry in a horizontal position to ensure that no herbicide contacted the soil. After treatments, plants were returned to the greenhouse for 6 d. The growth increment of the primary rhizomes was measured after excavating the plants. Bud viability was then bioassayed with 0.1% (w/v) of 2,3,5-triphenyl tetrazolium chloride.[15] The rhizomes were cut into sections containing one bud. Bud viability was visually evaluated by using a color index with 1 (white) corresponding to no viability and 5 (purple) to 100% viability. The results are reported as a percentage of the untreated control.

C. EXPERIMENT 3

The effect of ethylan and AMS added to glyphosate on quackgrass bud viability was assessed. The rate of herbicide used was 250 mg a.e. l^{-1}. Ethylan and AMS were used at 0.5% (v/v) and 5.0% (w/v), respectively. The same procedure as above was followed.

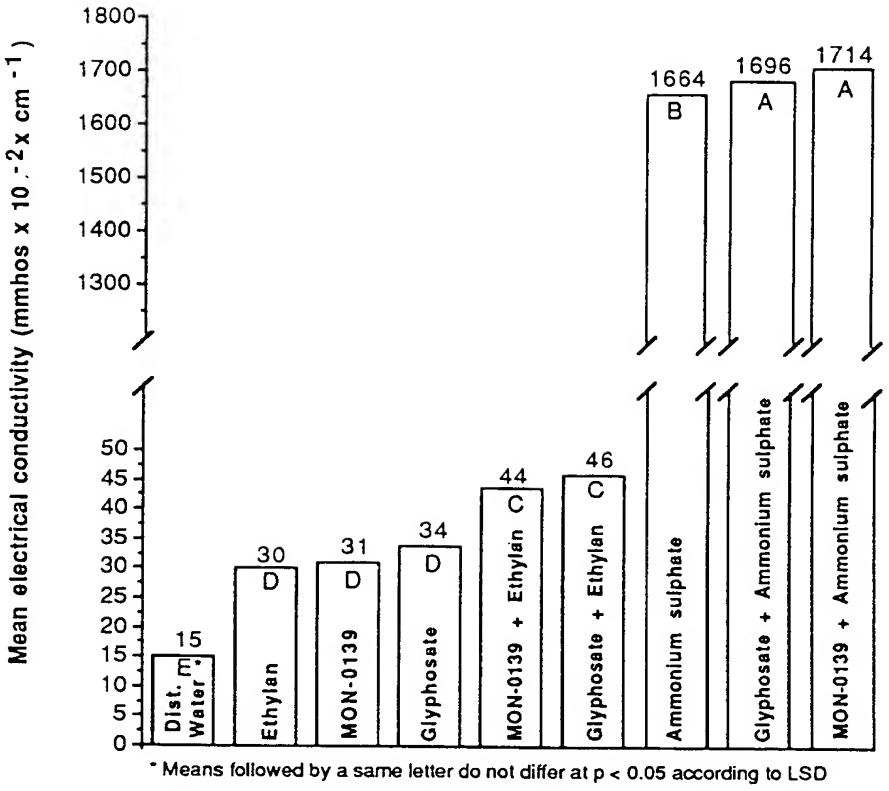

FIGURE 1. Effects on mean leaf-cell membrane permeability of ethylan and AMS added to glyphosate or MON 0139.

Each experiment used a completely randomized design with four replications. Each experiment was repeated twice and the data was submitted to standard analysis of variance after testing for homogeneity of variance. Treatment means were compared at $p \leq 0.05$ with the LSD (least significant difference) test. Regression analyses were performed to assess the influence of bud position on the response to herbicide treatments.

III. RESULTS AND DISCUSSION

A. EXPERIMENT 1

The addition of ethylan to either glyphosate or MON 0139 increased ($p < 0.05$) the mean leaf-cell permeability over that of glyphosate or MON 0139 (Figure 1). Mean leaf-cell permeability was increased severalfold by AMS over that of any other treatments. AMS increased the leaf-cell permeability of foliar discs treated with glyphosate and MON 0139. Any treatment resulted in greater leaf-cell permeability than the distilled water control.

Prendeville and Warren[11] have shown no effect of glyphosate on the leaf-cell permeability of bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L. [Merr.]) up to 12 h after treatment. They used a different procedure, but the rate of glyphosate used was similar. Plants were sprayed "to run off" and discs were punched from the leaves at different intervals, rinsed with, and incubated in distilled water. Conductivity of the ambient solution was then measured. While this procedure has the advantage of providing a common basis

for treatment comparison, it necessitates rinsing which results in solute leakage that is not taken into account in measurements.

The leaf-cell permeability reached a plateau about 4 h after treatment when no adjuvant was used with any of the herbicides (Figure 2). The permeability was constantly greater with glyphosate than with MON 0139. The mean electrical conductivity for foliar discs treated with herbicides was about twice that for untreated discs. The addition of ethylan to either herbicide increased the leaf-cell permeability, with a plateau at 16 h after treatment. The leaf-cell permeability reached a maximum (nearly 50 mmhos $\times$ 10^{-2} cm^{-1}) at 32 h after treatment for a mixture of any herbicide with ethylan, compared to 48 h with herbicide alone (nearly 40 mmhos $\times$ 10^{-2} cm^{-1}). These results indicate that ethylan increases the rate of herbicide penetration compared to herbicide alone.

The addition of AMS increased by about 50-fold the leaf-cell permeability of glyphosate and MON 0139. The leaf-cell permeability reached a plateau at 4 h for AMS and MON 0139 + AMS, and at 6 h for glyphosate + AMS (Figure 2). The leaf-cell permeability of foliar discs treated with MON 0139 was greater than that of discs treated with glyphosate. About 12 h after treatment, there was a steady decline in leaf-cell permeability when AMS was used. Growers have often noticed the rapid phytotoxic response of plants treated with glyphosate + AMS, but our results do no indicate any marked difference between treatments including AMS.

B. EXPERIMENT 2

Glyphosate reduced the mean bud viability of quackgrass rhizome to a greater extent than did MON 0139 (Table 1). The viability of glyphosate-treated buds was constantly less than that of those treated with MON 0139, regardless of the rate (Table 2). This result points out the usefulness of the surfactant added to the commercial formulation.

The position of buds on the rhizome had a marked effect on its viability following treatment (Figure 3). In all cases, herbicide-treated and untreated plants had increasing viability with increasing distance from the treated shoots. This result has been reported in the literature,[5,15] but the viability in untreated plants and those treated with 750 mg l^{-1} of MON 0139 increased at a greater rate than in those treated with glyphosate, with increasing distance from the treated shoot. While viability was lower than the 3.5 index value for all bud positions with glyphosate, the bud viability exceed that value for bud positions greater than 5 and 7 of untreated- and MON 0139-treated plants, respectively. The best-fit regression equations for bud viability (Y) in relation to bud position (B) are presented in Figure 3 for the 750 mg l^{-1} glyphosate treatment. The coefficients of determination R^2 ($p \leq 0.001$) varied between 0.71 and 0.84. Similar regression equations were obtained at 250 and 1250 mg l^{-1} rates (data not shown).

C. EXPERIMENT 3

The addition of ethylan or AMS increased by about 25% the phytotoxicity to quackgrass of solutions containing 250 mg l^{-1} of glyphosate (Table 3). Compared to the untreated control, the viability of glyphosate-treated buds averaged over 87%. In previous work, we have shown that no difference existed between glyphosate-treated and untreated buds when using tetrazolium for testing the viability of five quackgrass biotypes.[12] In contrast, bud viability was significantly reduced by glyphosate when buds were allowed to germinate on an agar medium. Great care should be taken when comparing the two methods. While the germination response to herbicide treatments may be more readily detected, total kill of the buds, as evaluated by using tetrazolium chloride, is not completed at the time of appraisal. Some studies have shown that ^{14}C-glyphosate tends to accumulate in the apical portion of quackgrass rhizomes.[3,6] Our results indicate that bud viability increased as buds were po-

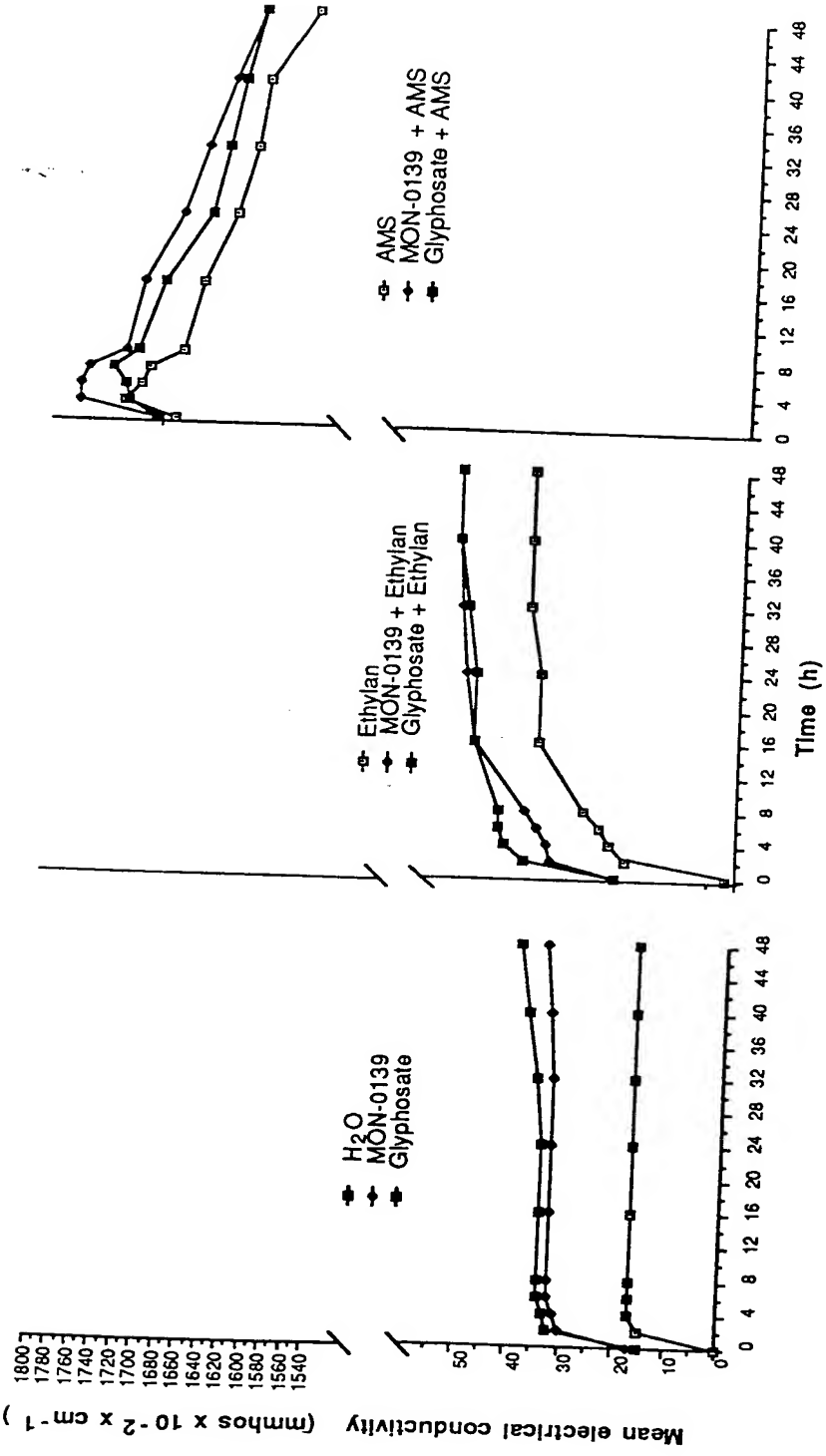

FIGURE 2. Time course of leaf-cell membrane permeability as influenced by ethylan and AMS added to glyphosate or MON 0139.

TABLE 1
Effect of MON 0139 and Glyphosate on Mean Quackgrass Bud Viability

Treatment	Mean bud viability (% of control)
MON 0139	88.1 a
Glyphosate	59.7 b

Note: Means followed by the same letter do not differ at $p < 0.05$ according to LSD.

TABLE 2
Dose Response of Quackgrass Bud Viability to MON 0139 and Glyphosate

Rate of a.e. (mg l^{-1} w/v)	Glyphosate	MON 0139
	(% of control)	
0	100 a	100 a
250	61 b	105 a
750	44 c	70 b
1250	47 c	67 b

Note: Means followed by the same letter do not differ at $p < 0.05$ according to LSD.

sitioned near the apex (Figure 3). While this may seem contradictory, glyphosate may accumulate in the apex and stimulate the respiratory activity of bud tissues, thus depleting their nutritive reserve at a faster rate. With time, a greater rate of kill is achieved in the apex of the rhizome rather than in the basal part.

Neither adjuvant modified the distribution of glyphosate among the rhizome buds, as reflected by a similar pattern of bud viability response (data not shown), but rhizome growth subsequent to glyphosate treatment was less with adjuvants than with glyphosate or no herbicide (Table 4). These results confirm those of Turner and Loader,[16] who have shown that both ethylan and AMS increased the phytotoxicity of glyphosate to quackgrass.

It is evident from our study that glyphosate modifies the leaf-cell permeability of quackgrass. Both adjuvants used have enhanced the penetration of glyphosate, thus favoring translocation toward the rhizomes. In general, glyphosate penetration was prolonged in the presence of an adjuvant, as solute leakage reached a plateau at a later stage than in the absence of an adjuvant. As a result, the growth increment following treatment with glyphosate was inferior when adjuvants were added to the herbicide solution. The usefulness of the adjuvant added to formulated glyphosate was demonstrated by a greater reduction of bud viability compared to unformulated glyphosate (MON 0139).

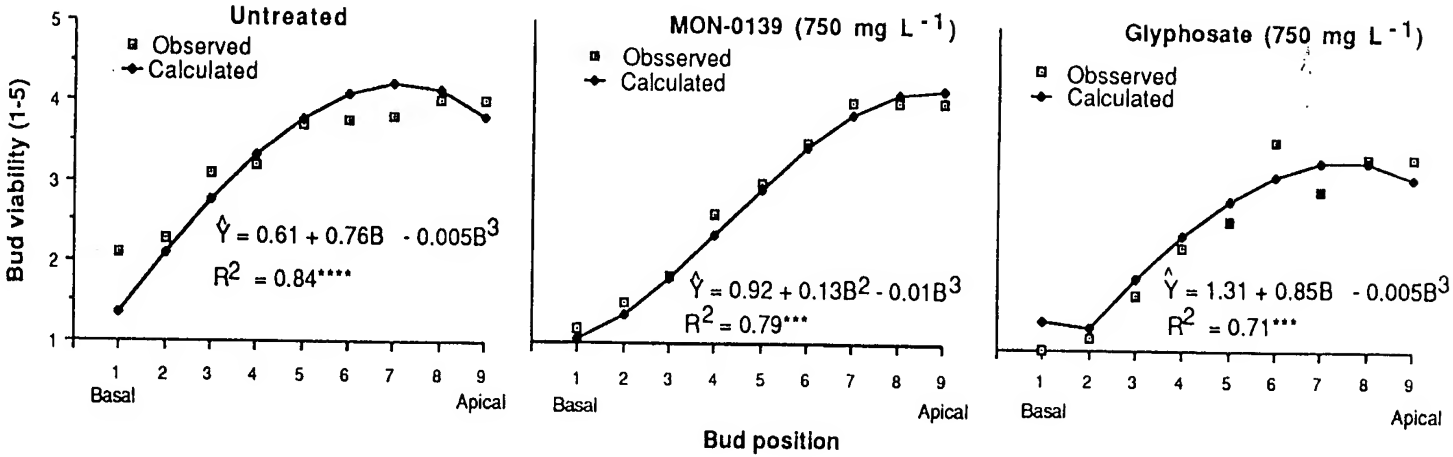

FIGURE 3. Effects of glyphosate and MON 0139 on bud viability as influenced by position on rhizome.

TABLE 3
Effect on Mean Bud Viability of Adding Ethylan or AMS to Glyphosate Applied at 250 mg l^{-1}

Treatment	Mean bud viability (% of control)
Glyphosate	87.5 a
Glyphosate + ethylan	66.0 b
Glyphosate + AMS	63.2 b

Note: Means followed by the same letter do not differ at $p < 0.05$ according to LSD.

TABLE 4
Effect on Rhizome Growth Increment after Adding Ethylan or AMS to Glyphosate Applied at 250 mg l^{-1}

Treatment	Rhizome growth (cm)
Control	7.64 a
Glyphosate	6.41 a
Glyphosate + ethylan	4.27 b
Glyphosate + AMS	2.62 b

Note: Means followed by the same letter do not differ at $p < 0.05$ according to LSD.

ACKNOWLEDGMENTS

The authors thank Mr. Jacques Debroux for his technical assistance. The products used were generously provided by Monsanto Canada and SDS-Biotech.

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Chapter 11

INFLUENCE OF ADJUVANTS ON CUTICULAR PENETRATION AND METABOLISM OF AMINOCARB FOLLOWING TOPICAL APPLICATION OF MATACIL® 180F FORMULATIONS TO SPRUCE BUDWORM

Kanth M. S. Sundaram

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ABSTRACT

Three mixtures, AID-585, AS-7N, and AA-3409, were prepared by mixing Matacil® 180F (a commercial formulation of aminocarb) with a light oil (insecticide diluent, ID 585), a heavy oil (Sunspray® 7N) or a surfactant (Atlox® 3409F)/water, respectively. The mixtures were fortified with ^{14}C-ring labeled aminocarb and were topically applied to fifth instar larvae of the spruce budworm. Penetration rates and depletion of the external residues were determined by ^{14}C-assay. The amount of aminocarb penetrated into the insects was determined by macerating them and extracting with ethyl acetate. The parent material and its metabolites present in the external rinses and in the extracts were determined by thin-layer-chromatography and a liquid scintillation counter. Penetration rates of aminocarb in the three mixtures varied according to the type of adjuvants present in them. The increasing order of penetration was AID-585 > AS-7N > AA-3409. The metabolites found, in decreasing order of concentration, were 4-methylamino, 4-amino, and 4-methylformamido aminocarbs. Trace levels of 4-formamido aminocarb, and 4-dimethylamino and 4-methylamino 3-methylphenols, were tentatively identified. The amount of aminocarb and its metabolites found varied according to the type of adjuvants and the rate of penetration.

I. INTRODUCTION

Aerial application of insecticides to control spruce budworm, *Choristoneura fumiferana* (Clem.), a widely distributed and most destructive defoliator of coniferous forests in eastern North America, is a well-established pest management strategy in Canada.[13] Aminocarb (trade name, Matacil®, 4-dimethylamino-3-methylphenyl *N*-methylcarbamate), a broad-spectrum contact insecticide, has been used since 1970 to control budworm populations in forests.[16] It is hypothesized that the mode of entry of sprayed toxicant is either due to (1) direct impingement of droplets on insects, (2) indirect contact with contaminated webbing within the microhabitat, or (3) ingestion of contaminated needles by budworm.[9]

Aminocarb kills budworm by virtue of being a cholinesterase inhibitor.[2] Topical application of the chemical showed that it is extremely toxic to insects.[15] It is postulated[3] that contact insecticides generally spread over the insect cuticle, enter the insect by crossing the integument, dissolve in the hemolymph, and eventually cause death. Assuming that penetration through the integument is the mechanism of entry of aminocarb into the insect, it is not yet clear (1) whether the penetration rate is influenced by the adjuvants present in different forestry spray mixes and (2) how rapidly the penetrated and surface residues are degraded in the insect. The toxicity of various aminocarb spray mixes is not only a function of the active ingredient (a.i.), but also of the adjuvants present in them.[7,8,20] Therefore, this study was undertaken with two objectives in mind: (1) to determine the effect of adjuvants on the cuticular penetration of topically applied aminocarb in fifth-instar budworm larvae and (2) to study the *in vivo* degradation and metabolism of the chemical in the insect.

II. MATERIALS AND METHODS

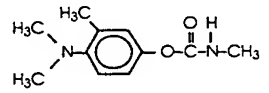
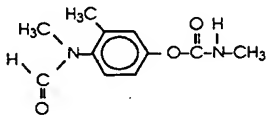
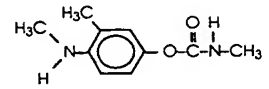
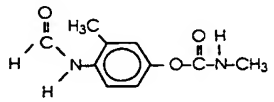
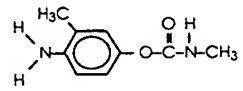
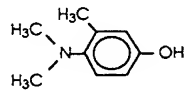
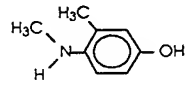
A. CHEMICALS

The radioactive aminocarb labeled with ^{14}C in the aryl ring ($^{14}C_{Ar}$-O-) (specific activity 11.7 mCi/mmol and >99% purity), analytical-grade aminocarb and its metabolites (purity >97%) listed in Table 1 were supplied by Mobay Chemical Corp., Kansas City, MO.

All organic solvents used were pesticide grade from Caledon, Georgetown, Ontario, Canada. The scintillation cocktail (Instal-Gel®) and the $^{14}CO_2$ absorber (Carbosorb®) were obtained from United Technology Packard. Anhydrous sodium sulfate (Fisher) was heated overnight at 260°C prior to use.

TABLE 1

Aminocarb and Some of Its Metabolites Found in Spruce Budworm Rinses and Homogenates with Their Structural Formulas and Corresponding R_f Values in Isopropyl Ether: Acetonitrile (4:1, v/v) Solvent System

NAME	STRUCTURAL FORMULA	ABBREVIATION	R_f
4- Dimethylamino-3-methylphenyl N-methylcarbamate		A	0.75
4- Methylformamido-3-methylphenyl N-methylcarbamate		MFA	0.24
4- Methylamino-3-methylphenyl N-methylcarbamate		MAA	0.64
4- Formamido-3-methylphenyl N-methylcarbamate		FA	0.20
4- Amino-3-methylphenyl N-methylcarbamate		AA	0.41
4- Dimethylamino-3-methylphenol		DAP	0.81
4- Methylamino-3-methylphenol		MAP	0.79

B. FORMULATIONS

The three formulations used in the study along with their abbreviations, compositions, and some key physical properties are given in Table 2. The suppliers of the formulation components are given in the footnotes. The Matacil® 180F formulations, AID-585 (oil solution), and AA-3409 (aqueous emulsion) are currently registered for forestry use in Canada, whereas the experimental formulation AS-7N (oil suspension) is prepared from the oil-soluble concentrate, Matacil® 1.8D (which is currently phased out), containing nonylphenol.

A 1-ml aliquot of each formulation was prepared in volumetric flasks by mixing the required amount of the ingredients listed in Table 2, using either micropipettes or burettes. A standard stock solution of ^{14}C-aminocarb (sp activity 11.7 mCi/mmol) in ethyl acetate containing 0.05 g/ml was prepared to give 104×10^3 dps/50 μg/μl. To each formulation, 100 μl of the stock solution was added to give 9.46×10^3 dps/μl. The total aminocarb (labeled 4.5 μg/μl + unlabeled 47.4 μg/μl) concentration in the end-use formulation was 51.9 μg/μl. The volumetric flasks containing the formulations were tightly capped and shaken thoroughly for uniform concentration. Each flask was wrapped in aluminum foil to minimize possible photolysis and stored at 0°C until use.

TABLE 2
Composition (vol %) of Aminocarb (Matacil® 180 F) Formulations Used in the Study and Their Properties at 20°C

Formulation composition (vol %)	Abbreviation	Density (g/ml)	Viscosity (mPas · s)	Surface tension (mN/m)
Matacil® 180F[a] (26.7) + I.D.[b] 585 (73.3)	AID-585	0.837	4.00	29.1
Matacil 180F (26.7) + Sunspray®7N[c] (73.3)	AS-7N	0.884	30.5	31.1
Matacil 180F (26.7) + Atlox® 3409[d] (1.3) + distilled water (72.0)	AA-3409	1.011	2.42	30.3

[a] Matacil® 180F (trade name for aminocarb [19.5% by weight] containing air-milled particles of a.i. at 2- to 3-μm-diameter size suspended in oil) supplied by Chemagro Ltd., Mississauga, Ontario, Canada.
[b] I.D. insecticide diluent 585 — a refined light petroleum distillate supplied by Shell Canada Ltd. It distills at or below 308°C and is composed of alkylated benzenes and naphthalenes.[5]
[c] Sunspray®7N — a viscous, refined heavy petroleum distillate supplied by Sun Oil Co., Philadelphia, PA. It distills at about 410°C (30 mmHg) and is composed of paraffinic hydrocarbons.[19]
[d] Atlox® 3409F — alkene/aryl sulfonate emulsifier supplied by Atkemix, Inc., Brantford, Ontario, Canada.

C. BUDWORM LARVAE

Budworm larvae were reared on an artificial diet at the Insect Production Unit of this institute and supplied to the study. Insects of uniform size (length, 2.1 ± 0.4 cm; mass, 0.012 ± 0.005 g) were selected and used in the experiment. Ten larvae per test and three replicates for each time period and formulation were used. When variability was large, more replicates were added.

D. APPLICATION OF AMINOCARB

Each aminocarb formulation was applied topically to budworms using a calibrated B. D. Yale-G498 syringe attached to an ISCO-Microapplicator (Model M). Sets of ten insects were taken in Teflon® Oak Ridge*-type centrifuge tubes (Nalgene cat. no. 3114-0010) and ten 1-μl droplets of about 1250-μm diameter were added to each set. The tubes were tightly sealed and shaken (Sybron Thermelyne Maxi Mix®) to ensure uniform coating of the chemical on the insects. During and after insecticide application, the larvae were kept in an environmental chamber maintained at 15 ± 1°C and 80 ± 3% relative humidity under simulated sunlight (400 W multivapor discharge lamps).

E. EXTRACTION PROCEDURE

At 0, 15, 30, 45, 60, 90, 120, 150, and 180 min posttreatment, aminocarb remaining outside the insect was removed by washing the larvae in the centrifuge tube twice with 5 ml of ethyl acetate. The pooled washings were dried through a column of anhydrous sodium sulfate, evaporated under vacuum to a known volume, and aliquots used for liquid scintillation counter (LSC) and thin-layer chromatography (TLC).

Aminocarb that had penetrated into the insect was quantified by homogenizing the washed insects in a Sorvall Omni-Mixer® (DM-2000) with 3 × 5 ml of acetonitrile and filtering under suction using a Millipore® filter. The pooled extract was passed through an anhydrous sodium sulfate column, concentrated under low pressure, and partitioned with 5 ml of hexane. The acetonitrile and hexane layers were gently flash evaporated to dryness and the residues taken in ethyl acetate for LSC and TLC studies.

* Teflon is the registered trademark of E. I. du Pont de Nemours and Company, Inc., Wilmington, DE.

TABLE 3
Penetration Rate and Percent Recovery of ^{14}C-Activity in Different Budworm Fractions After Topical Application of ^{14}C-Aminocarb Formulations

Time after application (min)	Average % activity in different fractions*				
	EtOAc rinse	CH_3CN layer	Hexane layer	Bound ^{14}C	Total
Formulation: AA-3409					
0	97	2	0.3	0.2	99.5
15	92	6	0.2	0.2	98.4
30	86	12	0.3	0.2	98.5
45	79	21	0.5	0.3	100.8
60	69	27	0.6	0.5	97.1
90	64	33	0.5	0.8	98.3
120	62	32	0.4	0.7	95.1
150	60	33	0.6	0.8	94.4
180	60	33	0.5	0.8	94.3
Formulation: AID-585					
0	90	10	0.2	0.0	100.2
15	70	29	0.1	0.2	99.3
30	52	47	0.3	0.2	99.5
45	44	53	0.2	0.3	97.5
60	30	64	0.2	0.2	94.4
90	27	65	0.3	0.3	92.6
120	27	66	0.3	0.4	93.7
150	25	65	0.2	0.3	90.5
180	24	64	0.3	0.4	88.7
Formulation: AS-7N					
0	93	7	0.2	0.0	100.2
15	81	17	0.3	0.0	98.3
30	70	28	0.2	0.0	98.2
45	61	38	0.2	0.2	99.4
60	49	48	0.4	0.3	97.7
90	46	51	0.2	0.2	97.4
120	47	50	0.4	0.3	97.7
150	44	52	0.3	0.2	96.5
180	45	50	0.3	0.4	95.7

* Values represent the mean for three replicate measurements. Standard deviations were less than 15% (not shown).

The unextractable ^{14}C-activity in the larval residue was estimated by combusting aliquots of it to $^{14}CO_2$ in a Packard Oxidizer-306 and counting in the LSC after absorbing the gas in Carbosorb®.

F. RESOLUTION OF AMINOCARB AND ITS METABOLITES

Aminocarb and its metabolites in the ethyl acetate wash and the acetonitrile phase of the larval extract following hexane partition were separated and analyzed by TLC.[18] The hexane phase was discarded due to its low level of radioactivity (<1%, AA-3409, range 0.2 to 0.6%; AID-585, range 0.1 to 0.3%; AS-7N, range 0.2 to 0.4%) (Table 3). Baker's

Si-HPF-7011-4 chromatoplates coated with a sorbent layer (200 μm) containing a mixture of 5 μm SiO_2 and 254 nm fluorescent indicator were used. Instead of the hexane-acetone (1:1, v/v) and diethyl ether-hexane-ethanol (77:20:3, v/v) solvent systems used earlier,[18] an isopropyl ether-acetonitrile (4:1, v/v) solvent system[10] was used in the present study to separate the parent material and its major metabolites (Table 1). Known nonradioactive compounds (Table 1) were chromatographed with aminocarb and its metabolites to help in identifying unknown products. The names of the compounds and their structural formulas, abbreviations, and R_f values obtained in this study are listed in Table 1. The fluorescent spots obtained for each compound on the chromatoplate were viewed under a UV lamp, and were further confirmed by radioautography.[17] The spots were carefully removed by scraping, transferred to scintillation vials containing Insta-Gel®, and the radioactivity determined with a Beckman LS-9000 liquid scintillation counter. The scrapings of the spots corresponding to all unidentified metabolites, including those remaining at the origin of the chromatoplate after solvent separation, were pooled, and its activity measured and recorded as unknown in Tables 4 and 5. No further attempts were made to either separate or identify the individual moieties present in them. The two phenols, DAP and MAP, were found in isolated cases and in very low amounts (about 0.5%) in the insect rinses (Table 4) and homogenates (Table 5). Their R_f values (Table 1) were very close and their individual quantification was not possible. Consequently, their combined activities, rather than their individual values, are recorded in Tables 4 and 5.

III. RESULTS AND DISCUSSION

A. CUTICULAR PENETRATION OF ^{14}C-AMINOCARB

Rates of penetration for the three formulations, expressed as percent activity in the three solvent fractions, are given in Table 3. The dosages used in the study were manyfold higher than the LD_{50} value (1.3 μg per insect for western spruce budworm, *Choristoneura occidentalis* Freeman),[15] which resulted in death of the insects within 20 to 25 min after application. The data (Table 3) clearly show the difference in the rate of penetration of radioactive aminocarb through the integument of the budworms. The formulation AID-585, containing ID 585 (low-boiling petroleum distillate composed of aromatics), penetrated most, and the formulation AS-7N, having less-volatile long-chain paraffinic hydrocarbons as solvent, penetrated moderately. In striking contrast, the emulsion formulation AA-3409 penetrated least. For AID-585, the activity in ethyl acetate rinses decreased exponentially with time, from the initial 90% to a low value of 27% 90 min after application. The corresponding values for AS-7N and AA-3409 were 93 and 46% and 97 and 64%, respectively, indicating that the adjuvants in the formulations exerted great influence on the penetration of the chemical. The increasing order of penetration observed in the present study is AID-585 > AS-7N > AA-3409. Beyond 90 min, the activity remaining on the cuticle leveled off, reaching nearly a constant value of 27% for AID-585, 46% for AS-7N, and 64% for AA-3409.

The increase and accumulation of radioactive aminocarb in the insect homogenates were exponential with time in all three formulations (^{14}C-activity in CH_3CN layer in Table 3). For AID-585, the activity increased rapidly from the initial 10% to 65% within 90 min after treatment and then reached a plateau. Similar trends were also observed for the other two formulations except that the 90-min peak levels were intermediate (51%) for AS-7N to low (33%) for AA-3409, confirming that cuticular penetration increased in the order of AID-585 > AS-6N > AA-3409 and that the oil carriers had exerted considerable influence on cuticular penetration compared to the emulsion formulation.

Insect cuticle is lipophilic due to the presence of lipids and hydrocarbons.[4] Lewis[6] reported that lipophilic solvents tend to spread rapidly over the surface of an insect under

TABLE 4
Degradation of ^{14}C-Aminocarb and Formation of Its Metabolites in Ethyl Acetate Rinses of Budworm

Time after application (min)	Percent of ^{14}C recovered from ethyl acetate rinses as indicated products*							
	A	MFA	MAA	FA	AA	DAP + MAP	Unknown	Total
Formulation: AA-3409								
0	97	0	0.5	0	0	0	0.5	98
15	93	0.5	1	0	0.5	0	1	96
30	91	0	2	0	1	0	3	97
45	90	0.5	2.5	0	1	0	3	97
60	86	1	4	0	2	0	4	97
90	80	1	3.5	0	3	0.5	7	95
120	77	0.5	2	0	2	0.5	9	91
150	73	0	2.5	0	1.5	0	12	89
180	70	0.5	1.5	0	2	0	16	90
Formulation: AID-585								
0	96	0	0	0	0	0	0	96
15	93	0	0.5	0	0.5	0	0	94
30	88	0	2	0	1	0	2	93
45	85	0.5	2	0	0.5	0	2	90
60	84	0.5	2.5	0	1	0	4	92
90	82	0	3	0	2	0	3	90
120	81	1	4	0	2	0	5	93
150	76	1	3	0	2	0	7	89
180	76	0	2	0	2	0	8	88
Formulation: AS-7N								
0	98	0	0	0	0	0	0	98
15	95	0	0.5	0	0	0	0	95.5
30	94	0	0.5	0	0.5	0	1	96
45	93	0	1	0	0.5	0	0.5	95
60	90	0.5	2	0	0.5	0	2	95
90	87	0.5	3	0	1.5	0	3	95
120	88	0	2	0	2	0	4	96
150	85	0.5	1.5	0	1	0	6	94
180	84	0	2	0	1	0	6	93

* Values represent the mean for three replicate measurements. Standard deviations were less than 15% (not shown).

the influence of interfacial forces, facilitating penetration of solutes by intercalation in the wax layers, whereas aqueous solutions, being poor wetters of cuticle, do not penetrate cuticular layers rapidly. Aminocarb is moderately lipophilic (K_{ow} = 58 ± 6 at pH 7),[10] and if formulated in lipophilic solvents such as ID 585 and Sunspray® 7N (Table 2), it has a greater tendency to penetrate the hydrophobic insect cuticle and concentrate inside the body than when formulated as an emulsion.

Plots of the percent ^{14}C-activity (Y) in ethyl acetate washes of insects (Table 3) vs. time (t) decreased curvilinearly and obeyed the exponential equation:

$$Y = A + Be^{-kt}$$

TABLE 5
Degradation of ^{14}C-Aminocarb and Formation of Its Metabolites in Acetonitrile Phase of Budworm Homogenate

Time after application (min)	Percent of ^{14}C recovered from acetonitrile phase as indicated products[a]							
	A	MFA	MAA	FA	AA	DAP + MAP	Unknown	Total
Formulation: AA-3409								
0	94	0	0.5	0	0	0	3.5	98
15	69	1	14	1	4	0	5	94
30	58	4	18	1	9	0	7	97
45	51	2	19	1	11	0	11	95
60	49	2	16	1	12	0	13	93
90	44	1	12	0	13	0	17	87
120	42	2	8	0.5	12	0.5	19	84
150	43	2	4	0	6	0	26	81
180	44	1	5	0.5	2	0.5	25	78
Formulation: AID-585								
0	96	0	0.5	0	0	0	1.5	98
15	75	0.5	9	0.5	3	0	4	92
30	64	2	15	1	5	0	6	93
45	60	1	16	0.5	7	0.5	9	94
60	53	1	14	1	9	0	11	89
90	50	0.5	7	0.5	10	0	15	83
120	51	1	5	0.5	7	0.5	17	82
150	49	0	6	0	6	0	18	79
180	52	0.5	3	0.5	3	0	20	79
Formulation: AS-7N								
0	96	0.5	0.5	0	0	0	1	98
15	82	1	8	0	2	0	3	96
30	76	1	11	1	4	0	6	99
45	70	0.5	13	0.5	7	0	7	98
60	67	1	13	0	8	0	7	96
90	63	0.5	10	0.5	6	0	12	92
120	64	1	7	0	4	0	13	89
150	63	0.5	4	0.5	4	0	15	87
180	64	0.5	5	0.5	4	0	16	85

[a] Values represent the mean for three replicate measurements. Standard deviations were less than 15% (not shown).

where A represents the percent residual ^{14}C-activity remaining in ethyl acetate over time, B represents the percent activity penetrated, and K is the rate constant. Nonlinear regression analysis of the data (ethyl acetate rinses in Table 3) gave the values for A, B, K, and R^2 (coefficient of determination) for the three formulations and are recorded in Table 6. The value A (residual activity in ethyl acetate) for AID-585 is lower (23.0%) and increases to 43.0% for AS-7N and then to 59.2% for AA-3409, indicating that cuticular penetration is relatively high for AID-585 and decreases in the order AID-585 > AS-7N > AA-3409. Correspondingly, the value of B (penetrated activity) is high for AID-585 (66.9%) and gradually decreases to 49.8% for AS-7N, and further to 37.9% for AA-3409, confirming that better insecticide diffusion has occurred in aminocarb formulations containing the oil

TABLE 6
Penetration Characteristics of Formulated Aminocarb in Spruce Budworm Following Topical Application and Regression Coefficients A, B, and C of the Exponential Equation $Y = A + Be^{-Kt}$ for Ethyl Acetate Rinse

Formulation abbreviation	A (% residual activity on insect surface)	B (% residual activity penetrated in insect)	K (penetration rate constant)	$T_{1/2}$ (min) for 50% penetration	R^2 (%) (coefficient of determination)
AA-3409	59.2	37.9	−0.018	—	0.96
AID-585	23.0	66.9	−0.029	31.40	0.99
AS-7N	43.0	49.8	−0.025	78.17	0.98

carriers compared to emulsion. Between the two oil formulations, the values of A and B clearly demonstrate that the low-viscosity carrier oil ID-585 promoted the diffusion of aminocarb more through the insect cuticle compared to the high-viscosity Sunspray® 7N. The rate constant K, representing the rapidity of ^{14}C-activity penetrated, increases (becomes more negative) from −0.018 for AA-3409 to −0.029 for AID-585, confirming the role of lipophilic adjuvants in cuticular penetration. The half-life ($T_{1/2}$) is lower for AID-585 (31.40 min) than for AS-7N (78.17 min), confirming that low-viscosity oil adjuvants such as ID-585 are very effective in promoting penetration of the a.i. compared to the high-viscosity adjuvants such as Sunspray® 7N. The value of A (residual activity on the insect surface) for AA-3409 is 59.2% (Table 6); therefore, no $T_{1/2}$ value could be calculated for this formulation.

The average bound residues for AID-585, AS-7N, and AA-3409 were about 0.3, 0.2, and 0.5%, respectively, with a noticeable increase in value for AA-3409 from 0.2 to 0.8% with time. With oil formulations, such a definite increase was not apparent (Table 3). The average total recovery ranged from 100.2 to 88.7% for AID-585, 100.2 to 95.7% for AS-7N, and 100.8 to 94.3% for AA-3409. Considering the uncertainties and errors involved in the experimentation, the recoveries reported in Table 3 are considered as quantitative.

B. INFLUENCE OF PHYSICAL PROPERTIES ON CUTICULAR PENETRATION

The measured physical properties (density, viscosity, and surface tension) of the three formulations are given in Table 2. Except for viscosity, the other two properties of the formulations are nearly the same. Consequently, their contributions to surface migration and the spreading of the droplet on the insect cuticle would be the same for all three formulations. Surface migration and spreading of droplets are a function of surface tension and viscosity.[6] Solutions of low viscosity can spread quickly over insect cuticle under the influence of interfacial forces, facilitating penetration of active ingredients.[3,6] In addition to lipophilicity, the low viscosity of AID-585 contributed to the rapid penetration of the insecticide solute through the cuticle. Contrary to this, the poor penetration of the active material in AA-3409, although its viscosity is the lowest, is attributable to its poor wettability of the hydrophobic cuticle and meager solubility of the solute particulates (2 to 3 μm in size) in the emulsion media. The viscosity of the AS-7N formulation is the highest in the series, but the toxicant has penetrated through the cuticle to an appreciable extent (Table 3). Evidently, factors other than viscosity are involved. Sunspray® 7N is nonvolatile and functions as a carrier for aminocarb particulates. Since it is lypophilic, we can hypothesize that the solvent will diffuse, spread, and migrate into the lipids of the epicuticle, thereby disorganizing the wax layer and facilitating the transport of the solute by diffusion across the epicuticle. The rate of

intoxication for a contact toxin is directly related to the rate of its penetration through the exposed insect cuticle.[6] Therefore, the choice of adjuvant in formulating commercial spray mixes, their solvent properties, and a thorough understanding of their interacting processes that take place at the epicuticle are important to obtain maximum efficiency in spray operations.

C. METABOLIC FATE OF AMINOCARB

The aminocarb, its metabolites containing intact carbamate moieties (MFA, MAA, FA, and AA), and the two phenolic mixtures (DAP + MAP) found in the ethyl acetate rinses and acetonitrile layers over the study period are given in Tables 4 and 5, respectively. Excluding the unknowns, the major components were the parent material and the mono (MAA)- and di (AA)-N-demethylated products. The metabolites detected were similar to those found in other studies.[1,11,21] Low levels of MFA were found frequently in the rinses and homogenates, whereas FA was found in small amounts in the acetonitrile layer only (Table 5). The phenolic moieties (DAP + MAP) were found occasionally at very low levels (activity 0.5%) in both liquid phases. Although the amount of products found in the rinses and homogenates varied, the pattern and spectrum of products formed in both were similar. In order of decreasing concentration, the major metabolites in both liquid phases (Tables 4 and 5) were MAA, AA, MFA, and FA (FA was found frequently at low levels in the acetonitrile phase), with sporadic presence of the phenols (DAP + MAP).

The percent activity of aminocarb in the ethyl acetate layer (Table 4) decreased from 97 to 70 for AA-3409, from 96 to 76 for AID-585, and from 98 to 84 for AS-7N. Correspondingly, a gradual increase in the activities of MAA and AA were observed in all cases, reaching maximum values around 90 min, and thereafter, their activities decreased. Contrary to these, the activities of the unknowns gradually increased with time as chemical and enzymatic degradations continued. The activity levels of the metabolites, and the unknowns formed, varied according to the formulation type.

The percent ^{14}C-activity of aminocarb recovered from the acetonitrile phase (Table 5) decreased with time, from 94 to 44 for AA-3409, from 96 to 52 for AID-585, and from 96 to 64 for AS-7N. The decrease is probably due to the increased metabolic activity with time. Apart from four- to fivefold increases in the quantities of the identifiable metabolites (Table 1) and two- to threefold increases in the unknowns, the pattern of degradation of aminocarb and the formation of metabolites (increase in MAA and AA initially with time followed by their decrease, and increase of unknowns with time) in the homogenates were similar to those found in the cuticular rinses.

The occasional formation of small amounts (0.5%) of the phenolic moieties (DAP + MAP) in the rinses and homogenates indicate that hydrolysis of the cabamate ester bond is not a predominant reaction in this study, contrary to the observations made elsewhere.[12] Nothing is known about the identity of the unknown metabolites detected on the TLC plate, including the ones at the origin. We may speculate that some of them may be products formed by ring hydroxylation and deamination, and by oxidation of ring-amino (H_2N-Ar), ring-methyl, or carbamate methyl groups through the oxidative microsomal enzymes in the insect. The transformation products at the origin appear to be more polar, and they may be amino acid or glucosidic conjugate moieties containing phenolic, carboxylic, and amino groups. The formation of such conjugates has not been demonstrated in budworm, but such a possibility cannot be ruled out.

Attempts to obtain the mass balance for the data recorded in Tables 4 and 5, with the assumption that the activities of the ethyl acetate rinses and acetonitrile extracts (Table 3) are 100% each, were successful only for the surface wash wherein >88% of the assumed value was recovered. However noticeable deviations (only >78% recovery) occurred for

the extract, especially beyond 90 min. No possible explanation could be given for this discrepancy.

From the results presented in Tables 4 and 5 and from the foregoing discussions, the major degradative pathway of aminocarb in eastern spruce budworm appears to be successive oxidative N-demethylation of the dimethylamino group, first forming MFA, followed by the formation of the corresponding transient carboxylic acid and decarboxylation of the acid to yield MAA. Similar degradative steps of MAA would yield first FA and then AA. Hydrolysis of A and MAA would yield the corresponding phenols DAP and MAP. In the case of MAP, it is not known whether the hydrolysis of MAA preceded oxidation or vice versa. Apart from the parent material, the stable and most prominent degradation products in the study are MAA and AA. The sequence of transformation steps outlined above for aminocarb in the insect is somewhat speculative, but the pattern of products formed is in agreement with the fate of closely related compound, mexacarbate (which differs from aminocarb only in possessing a methyl group in ring position 5), in western spruce budworm.[14]

ACKNOWLEDGMENTS

The author thanks Mobay Chemical Corporation, Kansas City, MO for supplying the radioactive aminocarb and other analytical standards used in the study, and N. Boyonoski, R. Wing, and T. Gidding for technical assistance.

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Chapter 12

THE CHARACTERIZATION OF UPTAKE AND TRANSPORT WITH A RADIOLABELED ARYLOXYPHENOXYPROPIONATE HERBICIDE AS INFLUENCED BY ADJUVANTS

Robert L. Noveroske, F. Nelson Keeney, and J. Grahm Brown

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ABSTRACT

In order to study the impact of various adjuvants on the uptake and translocation of aryloxyphenoxypropionate herbicides, high pressure liquid chromatography (HPLC) studies were conducted to compare the metabolic profiles from treated and untreated plant fractions of giant foxtail (*Setaria faberi* Herrm.) treated with a foliar application of ^{14}C-haloxyfop {2-[4-[[3-chloro-*s*-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propansic acid}. Similar metabolic profiles were found to exist between the treated leaf tissue and the export fraction.

Experiments were then conducted with various adjuvants being added to the standard formulation. Crop oil concentrate (COC) and crop oil (CO) were more effective in increasing uptake and transport than nonionic wetters. The combination of CO plus wetter resulted in the most efficient level of ^{14}C-uptake and -transport. Subsequent bioassay studies confirmed the benefit of CO-nonionic wetter blends in maximizing herbicidal activity.

The resultant enhanced biological response of the active ingredient permitted the design of formulations containing sufficient volumes of CO and wetter in the formulation concentrate itself, which on dilution with water in the spray tank did not require additional adjuvant. Use of radiotracer techniques are a highly effective means by which to quantitatively select adjuvants, wetters, and their combinations in order to define formulations with maximum performance potential.

I. INTRODUCTION

The biological activity of a postemergence systemic herbicide is dependent upon its physical and chemical properties, and interactive processes on and in the plant. The availability of the active ingredient at the active site is a function of its ability to penetrate the plant cuticle and the translocation and metabolic processes within the plant. The interaction of processes occurring on the plant surface — volatization, degradation, and penetration — and the resultant impact on active ingredient available for transport have been studied in detail.[10]

Esters of haloxyfop, such as haloxyfop-methyl and haloxyfop-ethoxyethyl, have been developed for postemergence grass control with excellent selectivity to soybeans and other broadleaf crops.[1,15,16] As with other aryloxyphenoxypropionates, the esters penetrate readily, followed by rapid hydrolysis to the acid,[5,7,10] the active ingredient in the plant.[3,13,14] Further metabolic activity may result in formation of conjugates within the plant which may act as a hydrolyzable source of active ingredient.[8,14]

Considerable effort has been spent studying the effect of adjuvants on increasing the activity of postemergence herbicides.[2-4,6,11,12,15] Among the variety of adjuvants tested, petroleum oils applied as tank-mix additives have been found to provide the most consistent levels of improved activity with aryloxyphenoxypropionates.[4,6,10] Tank-mix combinations of COC (1.25% v/v) and nonionic wetter (0.25% v/v) were found to be the most effective treatment for enhancing the activity of {3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carboxyl]amino]sulfonyl]-2-thiophencarboxylic acid}, a sulfonylurea herbicide, on *Kochia* sp. under stressed plant conditions.[12]

Uptake and transport studies with radiolabeled haloxyfop-methyl were initiated in an attempt to understand the influence of adjuvants on enhancing the mobilization of ^{14}C-activity into the plant, in order to optimize formulation design and behavior. This chapter summarizes the results of the impact of various adjuvant blends on formulation performance as measured by the uptake and transport of the ^{14}C-labeled active ingredient after foliar applications of radiolabeled haloxyfop-methyl on giant foxtail.

TABLE 1
Adjuvant Compositions and Manufacturers

Adjuvant	Manufacturer	Composition
Multifilm X-90	IWD, Ltd.	Polyoxyethylene (9) nonyl phenyl ether
Polyglycol 59-13	Dow Chemical U.S.A.	Polyoxyethylene (8) tridecyl ether
Atplus 411F crop oil concentrate (COC)	ICI Americas, Inc.	83–85% Paraffin oil and 15–17% nonionic emulsifier
Sunspray 11E crop oil (CO)	Sun Oil Co.	97% Paraffin oil and 3.0% nonionic emulsifier

The ultimate goal of this research was to gain sufficient insight on uptake phenomena so as to be able to design delivery systems which contained active ingredient and adjuvants in concentrations sufficient to provide the performance level and handling characteristics desired without having to resort to additional tank-mixing efforts.

II. MATERIALS AND METHODS

A. ADJUVANT STUDIES

A series of petroleum-based adjuvants and nonionic wetters were evaluated in the greenhouse to determine their potential to enhance the activity of haloxyfop-methyl and haloxyfop-ethoxyethyl for control of quackgrass (*Elytrigia repens* (L.) Nevski) and foxtails (*Setaria* spp.). A list of adjuvants, their composition, and corresponding manufacturer are shown in Table 1.

Seeds were planted in drainable, polyethylene pots (5 × 5 × 8 cm) containing Jiffy mix. Established seedlings were thinned to 0.1 to 0.2 plants per cm^2. All pots were watered with one-half strength Hoagland's solution as needed. the greenhouse was maintained at 25 ± 5°C with a light intensity of 550 $\mu E\ m^{-2}\ s^{-1}$ photosynthetically active radiation. Supplemental lighting maintained a photoperiod of 15 h.

Plants were grown to the three- to four-leaf stage (requiring approximately 14 d) before herbicide treatment. Plants were selected for uniformity and treated with foliar applications of formulated haloxyfop-methyl and haloxyfop-ethoxyethyl as emulsifiable concentrates (ECs). All treatments were applied in a spray chamber with an automated track sprayer operating at 4.8 km/h and 46 cm above the plant canopy with a T-Jet flat fan nozzle calibrated to deliver a total spray volume of 187 l/ha at 276 kPa. The soil surface was covered with vermiculite absorbent prior to treatment to prevent soil activity of the individual herbicides. It was removed after the spray treatments were dried.

Treatment replications were randomized and plants were watered with one-half strength Hoagland's solution by subirrigation as needed. Plants were graded for growth reduction relative to controls which included formulation inerts, using a scale of 0 to 100% (0 = no effect, 100 = total kill). GR_{80} values were calculated using linear regression analysis. The GR_{80} values reported reflect the amount of active ingredient needed, expressed as grams of acid equivalent (a.e.) per hectare, to provide 80% control.

B. RADIOTRACER STUDIES

Three- to four-leaf-stage seedlings (10 to 11 d old) of giant foxtail were used for all ^{14}C-studies. Plants were grown in a vermiculite-Jiffy mix blend (50:50) in drainable polyethylene pots (5 × 5 × 8 cm deep) under the same nutritional schedule, temperature, and light conditions as described for greenhouse bioassays. Experiments were conducted in an environmental chamber with a light intensity of 220 $\mu E\ m^{-2}\ s^{-1}$ and a temperature of 28°C. Plants were incubated in the chamber for 24 h before being treated.

The radiolabeled formulation was prepared by adding ^{14}C-haloxyfop-methyl-ph-UL-^{14}C with a specific activity of 19.98 mCi/mmol to the EC formulation blank. Treatments were prepared in crimp-top micro vials by adding the formulation to tap water (pH 8.2 to 8.4). Optionally, wetter and CO were added as dictated by the experiments.

Applications were made to the second, fully expanded leaf from the apex with a microsyringe at the rate of 140 g a.e./ha in 187 l of total spray volume. Additional replicates of leaf tissue were treated and immediately rinsed for 10 s in 5 ml of acetonitrile (ACN) in scintillation vials to determine zero-time application concentrations. Ten milliliters of Aquasol were added and the samples counted in a liquid scintillation counter (LSC). The ACN-rinsed leaves were combusted in a tissue oxidizer and the amount of $^{14}CO_2$ generated was determined using LSC techniques. Rinses and combustions were used collectively to establish the dose applied.

At desired time intervals in each experiment, four replications of each treatment were harvested. Treated leaves were removed, rinsed in ACN for 10 s to remove unabsorbed ^{14}C-activity, and counted using LSC techniques to establish the amount of ^{14}C-activity which had not penetrated the plant. The rinsed leaves were combusted in a tissue oxidizer and assayed for $^{14}CO_2$ using LSC techniques to establish the amounts of ^{14}C-activity associated with the treated leaf which had penetrated (leaf uptake minus transport). Plants were cut at the soil line, roots carefully removed from the potting medium, and combusted to reflect ^{14}C-basipetal transport. In a like manner, ^{14}C-activity associated with the untreated aboveground portion of the plant (untreated leaves) was determined. Leaf uptake plus transport values were used collectively to quantify uptake, the total fraction which had penetrated into the plant. Recoveries of ^{14}C-activity were expressed as a percent of the total amount applied to plants at zero time.

C. HPLC STUDIES

HPLC analyses were conducted using a Waters HPLC equipped with a 30.0-cm micro Bondapak C_{18} column. Twenty-four hours after treatment, leaves were removed and rinsed sequentially with ACN to remove surface residues. Treated and untreated plant portions were homogenized and extracted in 50% ACN, and 200-µl aliquots of these extracts were chromatographed and assayed by HPLC. The solvent system employed as 0 to 100% ACN with 1.0% acetic acid over 20 min, then held for 10 min. The flow rate was 1.5 ml/min, with eluent collected in 1.0-min fractions. HPLC chromatograms of ^{14}C-haloxyfop-methyl and haloxyfop were developed in this solvent system for reference purposes.

III. RESULTS AND DISCUSSION

Experiments were conducted on *Setaria* spp. to correlate biological activity with ^{14}C-uptake and transport patterns, and to study adjuvant effects resulting from postemergence applications of radiolabeled haloxyfop-methyl. Results of a greenhouse bioassay comparing the effectiveness of a standard EC formulation (XRM-4570), alone and tank mixed with COC (1.25% v/v), with that of a high emulsifier-containing formulation (XRM-4685) are summarized in Table 2.

Tank-mix applications of COC substantially improved the activity of XRM-4570. XRM-4685 containing high (50% w/w) emulsifier provided a measurable improvement in activity when compared with XRM-4570. Wetter concentrations from the formulations at their respective GR_{80} levels differed about fourfold. Adjuvant benefits from nonionic wetters are generally detectable at concentrations of 0.05% (v/v) and higher.[9] The results of this experiment are consistent with this observation. An experiment was conducted on *S. faberi* with radiolabeled haloxyfop-methyl as XRM-4570 and XRM-4685, both with and without

TABLE 2
Effect of Adjuvants on the Activity of Haloxyfop-Methyl for Control of *Setaria lutescens**

Treatment	Adjuvant conc % (v/v/187 l/ha)	GR_{80} (g a.e./ha)
XRM-4570[a]	0.016	89.79
XRM-4570 + 1.25% COC	1.250	33.64
XRM-4685[b]	0.063	56.22

* Dow Chemical U.S.A., 1985.

[a] 240 g a.e./l EC.
[b] 240 g a.e./l EC — high emulsifier content.

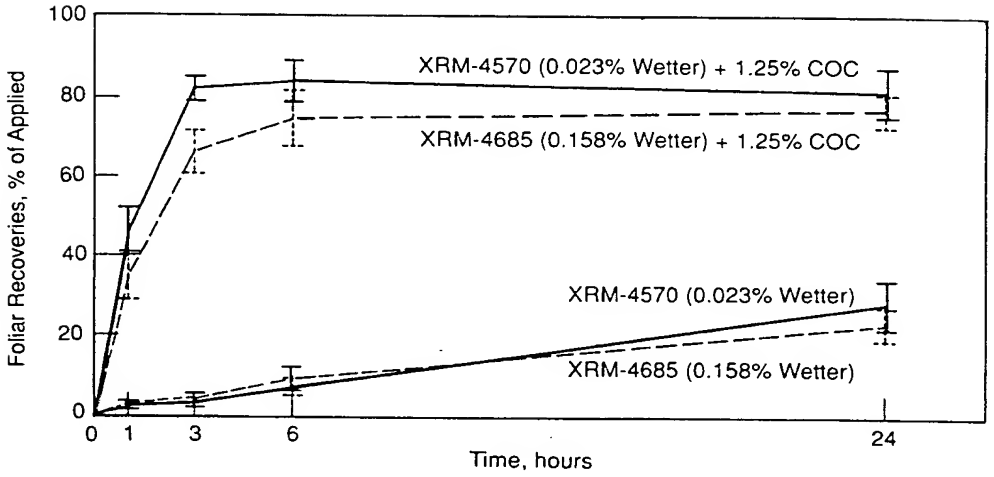

FIGURE 1. The effect of adjuvants on uptake of ^{14}C-activity from leaves of *S. faberi* treated with haloxyfop-methyl (140 g ae/ha).

tank mixes of COC (1.25%, v/v), to determine the impact of formulation and adjuvants on ^{14}C-uptake. The results are summarized in Figure 1.

Applications of COC markedly enhanced foliar uptake of ^{14}C-activity from both formulations. Differences due to wetter concentration between the two formulations were not detected, as was the case in the bioassay (Table 2). Lack of agreement is not believed due to species differences, since earlier work had shown no differences in ^{14}C-uptake patterns between the two formulations with and without COC when tested on *S. faberi* and *S. lutescens*.[9]

A possible explanation may be the impact of the increased deposition on the leaf surface when the composition was applied with a track sprayer, compared with radiotracer applications which utilized a microsyringe to apply a few droplets to the plant leaf. Alternatively, uptake may not be the rate-limiting process which can be used to judge formulation behavior in bioassays.

An experiment was conducted to determine the metabolic profiles of ^{14}C-activity from treated vs. untreated portions, so as to establish a relationship between the nature of the ^{14}C-residues in treated vs. transported plant fractions. HPLC chromatograms from extracts

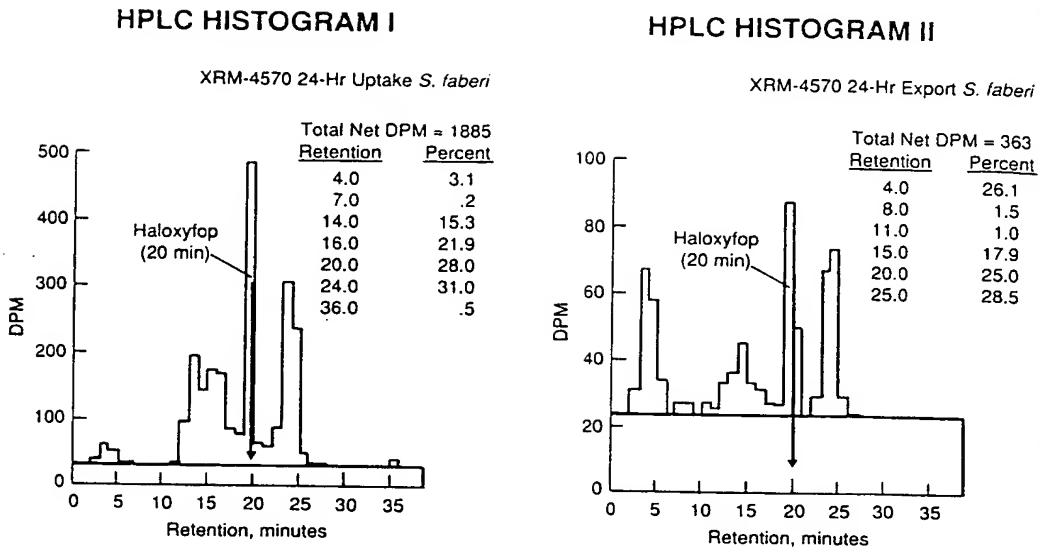

FIGURE 2. HPLC profiles of radioactivity from extracts of treated (I) and untreated (II) plant tissue of *S. faberi* 24 h after a foliar application of haloxyfop-methyl as XRM-4570 at 140 g ae/ha in 187 l of water.

of treated and untreated plant tissue of *S. faberi* were developed 24 h after a foliar application of ^{14}C-haloxyfop-methyl as XRM-4570 at 140 g a.e./ha in 187 l of water (Figure 2).

Peaks with a retention time identical to that of haloxyfop (20 min), the active ingredient in the plant,[3,13,14] were found in approximately similar concentrations in the extracts, along with several metabolites proposed to be conjugates.[10] No parent ester was detected, consistent with the findings that aryloxyphenoxypropionate esters are rapidly hydrolyzed by the plant.[5,7,10]

From these results, it was concluded that a similar metabolic profile exists between treated leaf tissue and export fractions and that, depending upon the experimental objectives and sensitivity needed, total ^{14}C-activity from either fraction could be useful in quantifying the benefits of adjuvants in studying formulation behavior.

An experimental 120 g a.e./l of EC formulation of haloxyfop-methyl containing 25 wt% each of nonionic emulsifier and CO was prepared which was found to exhibit acceptable handling properties. A radiolabeled sample was prepared and ^{14}C-transport compared with ^{14}C-haloxyfop-methyl as XRM-4570, with and without a tank mix of COC (Figure 3).

Levels of ^{14}C-transport with the experimental formulation were similar to those realized by XRM-4570 plus 1.25% COC, despite the fact that CO-wetter concentrations at finished dilutions were appreciably lower (0.07% each, 0.14% total) than contained in the XRM-4570 + COC treatment (0.012% wetter + 1.25% COC). This suggests that ratios of wetter and oil may exist which may improve the performance of an active ingredient, allowing the use of appreciably lower levels of oil than conventionally used in tank-mix practices.

A greenhouse bioassay was conducted in which a dilution series of haloxyfop-ethoxyethyl was bioassayed with a dilution series of CO as Sunspray® 11E and the wetter Polyglycol 59-13, each alone and in combination (Table 3).

At the high (50 g a.e./ha) rate, good adjuvant benefits resulted from each of CO and wetter independently, when compared to the no-adjuvant treatment. At the median (25 g a.e./ha) rate, control of *Elytrigia repens* was markedly enhanced by combinations of CO and wetter. Concentrations of 12.5 g a.e./ha were too low to detect any adjuvant benefits.

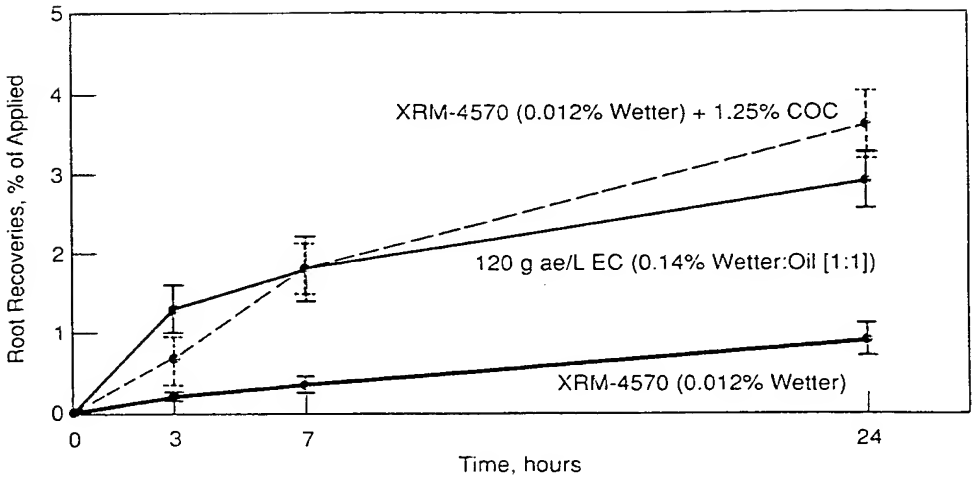

FIGURE 3. The effect of adjuvants on basipetal transport of ^{14}C-activity from leaves of *S. faberi* treated with haloxyfop-methyl (70 g ae/ha).

TABLE 3
Effect of Adjuvants on the Activity of Haloxyfop-Ethoxyethyl for Control of *Agropyron repens**

Treatment	Adjuvant Conc. % (v/v)/300 l/ha	% control 22 DAT, g a.e./ha 0.0	12.5	25.0	50.0
EF-646[a] +					
No Adjuvant			0.0	0.0	0.0
Sunspray 11E	0.125		5.0	0.0	100.0
	0.500		0.0	5.0	100.0
	1.000		0.0	25.0	100.0
PG 59-13	0.010		0.0	0.0	30.0
	0.050		0.0	25.0	100.0
	0.100		0.0	40.0	100.0
Sunspray 11E + PG 59-13	0.125 ± 0.010		0.0	65.0	100.0
	0.500 + 0.010		0.0	65.0	100.0
	0.125 ± 0.050		0.0	100.0	100.0
	0.500 ± 0.050		30.0	95.0	100.0
Untreated		0.0			

* Dow Chemical Europe, King's Lynn, 1984.

[a] 104 g a.e./l EC.

A field experiment was conducted comparing the adjuvant effects of oil, wetter, and oil-wetter combinations on haloxyfop-ethoxyethyl for control of large crabgrass [*Digitaria sanguinalis* (L.) Scop.] (Table 4). The results of this experiment support the ^{14}C-transport conclusions (Figure 3), confirming the benefit of CO-wetter adjuvant combinations and their effectiveness in maximizing activity.

Based upon the above experimental results, it is concluded that the use of radiotracer techniques are of value in quantifying the effects of adjuvants on the uptake and transport

TABLE 4
Effect of Adjuvants on the Activity of Haloxyfop-Ethoxyethyl for Control of *Digitaria sanguinalis**

Treatment	Adjuvant Conc, % (v/v)/200 l/ha	% brownout (150 g a.e./ha) trial I — 70 DAT	% brownout (150 g a.e./ha) trial II — 56 DAT
XRM-4638[a] +			
Sunspray 11E	1.000	90.0	85.0
Multifilm X-90	0.300	65.0	73.0
Sunspray 11E + Multifilm X-90	0.150 + 0.150	85.0	90.0
	0.200 + 0.100	72.0	80.0
	0.225 + 0.075	85.0	85.0
CV		10.0	10.0

* Dow Chemical Pacific, New Zealand, 1986.

[a] 240 g a.e./l EC.

of ^{14}C-activity in plants from postemergence application of radiolabeled herbicides. When used in conjunction with bioassays, such techniques represent a powerful research tool by which to characterize the performance of an active ingredient, study formulation behavior, and optimize delivery systems.

ACKNOWLEDGMENTS

The authors thank J. W. Brenkley, M. J. Watson, and P. J. Dryden of DowElanco, Pacific for the use of their field data.

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Chapter 13

ACTIVATION OF THE FOLIAR UPTAKE OF TWO WATER-SOLUBLE COMPOUNDS BY ALCOHOL POLYOXYETHYLENE SURFACTANTS

David Stock, Peter J. Holloway, Paul Whitehouse, and B. Terrence Grayson

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ABSTRACT

The influence of four C_{13}/C_{14} fatty alcohol surfactants with mean ethylene oxide contents of 6, 11, 15, and 20 on the foliar uptake of ^{14}C-labeled solutions of methylglucose and phenylurea by field (broad) bean (*Vicia faba* L.) and wheat (*Triticum aestivum* L.) was investigated under controlled environment conditions. Surfactant concentration and ethylene oxide content were found to influence greatly the uptake of the two compounds. Uptake activation only occurred when a threshold concentration of surfactant was exceeded. Uptake of ^{14}C-methylglucose was greatest in the presence of surfactants of high ethylene oxide content, while that of ^{14}C-phenylurea was much less sensitive to surfactant structure. Marked differences in the amount of movement of radiolabel were observed between field bean and wheat following applications of ^{14}C-phenylurea formulations, but these could not be ascribed to the properties of the surfactants added.

I. INTRODUCTION

Surfactants are used routinely as spray adjuvants to enhance the field performance of pesticide formulations. However, while the uptake and performance-promoting properties of nonionic surfactants have been exploited in commercial pesticide formulation, remarkably little is known about how this activation is achieved. Some research, however, has provided circumstantial evidence for specific mechanisms of activation, such as copenetration of surfactant with the active ingredient[9,13] and a humectant action of the surfactant.[12,13]

It is our view that a better understanding of structure-activity relationships is needed as a basis for detailed studies of the mechanisms of surfactant-induced activation of foliar uptake. There is very little information in the open literature dealing systematically with effects of either surfactant structure or concentration on the foliar uptake of agrichemicals; many reports describe the influence of a random selection of nonionic, cationic, and anionic compounds with little attention being paid to concentration. Some more extensive surveys have been conducted, however, into the enhancement effects of nonionic surfactants on the activity of a number of herbicides.[5,10,15]

Factorially designed radiochemical uptake studies have already demonstrated the importance of the physicochemical properties of the penetrating molecule in influencing the optimal surfactant structure needed for its activation.[4] The object of this chapter is to illustrate the importance of both surfactant structure and concentration in enhancing the uptake of foliage-applied compounds. It describes work on the uptake-promoting properties of a series of fatty alcohol polyoxyethylene surfactants when added to formulations of two model water-soluble compounds. Parallel work at Long Ashton Research Station (LARS) with similar, radiolabeled surfactants is directed at establishing the exact mechanism(s) involved in foliar uptake activation by this class of nonionic surfactant.

II. MATERIALS AND METHODS

A. PLANT MATERIAL

Two plant species were used for uptake studies. Field bean (cv. Maris bead), which has leaves with little epicuticular wax, was selected to contrast with wheat (cv. Minarette), which has a microcrystalline, water-repellent deposit of foliar wax.

All plants were raised from seed and grown in 9-cm pots of Arthur Bower's peat-based compost. After initial propagation in a greenhouse, plants were transferred to a controlled environment (CE) room at least 2 weeks before experimentation. Field bean plants were

used for uptake experiments 3 weeks after sowing, and wheat plants after 4 weeks. The CE room provided a 20°C (light)/15°C (dark) temperature regime with a 16-h photoperiod of 300 μmol m^{-2} s^{-1} provided by fluorescent tubes supplemented with tungsten lamps. The humidity of the CE room was not regulated, but followed a regular diurnal pattern of 71 to 81% relative humidity (RH) during the light period and 88 to 93% RH in the dark.

B. ALCOHOL POLYOXYETHYLENE SURFACTANTS

This surfactant class is frequently used in pesticide formulations. Four members of the Marlipal 34 series (Hüls, Marl, Germany), with mean molar ethylene oxide (E) contents of 6, 11, 15, and 20, and hydrophile-lipophile balance (HLB) values of 11.2, 14, 15, and 16.2, respectively, were selected. The parent primary alcohol (A) for the series is a C_{13}/C_{14} mixture, with straight-chain compounds accounting for 45% of the total. The remainder consists of methyl branched homologues.

C. WATER-SOLUBLE MODEL COMPOUNDS

Two radiolabeled compounds were investigated: 3-*O*-methyl-α-D-glucose and phenylurea.

Methylglucose is a highly water-soluble monosaccharide with an estimated log P (octanol/water partition coefficient) of −3.0. The 3-*O*-methyl-D-[U-^{14}C]glucose (50 μCi/mmol) was obtained from Amersham.

Phenylurea (log P 0.8) is structurally related to a number of herbicides and is much less water soluble (4086 ppm) than methylglucose. ^{14}C-carboxyl-labeled phenylurea was synthesized at LARS and had a specific activity of 11.5 μCi/mg.

D. EXPERIMENTAL DESIGN

A 4 × 3 factorial design was used, with the four selected surfactants at concentrations of 0.02, 0.1, and 0.5% (w/v). Test compounds were added to each formulation at a fixed concentration of 0.05% (w/v); a control treatment without any added surfactant was also included. Four replicates were used per treatment at each sampling interval.

E. PREPARATION AND APPLICATION OF TREATMENT SOLUTIONS

Treatment solutions (100 μl) were prepared in angle-bottomed vials[2] 1 h before each experiment. All treatment solutions contained about 5000 dpm/μl and were formulated in acetone-water (1:1) to ensure comparability with other investigations at LARS involving compounds of low water solubility.[4] Other workers[11] have established that the presence of a volatile organic solvent does not significantly influence the foliar uptake process.

Droplets were applied to leaves using a Burkard PAX-100 Programmable Microapplicator fitted with a 50-μl syringe.[2] Applications were performed inside the CE room, as were subsequent samplings. Treatment solutions were applied as 10 × 0.2-μl droplets to the central region of the adaxial surface of the third leaf of field bean plants and to the same region of the youngest fully expanded leaf of wheat plants.

F. DETERMINATION OF UPTAKE

Uptake was determined at various time intervals after application of radiolabeled formulations. Suitable sampling intervals were selected from the results obtained from preliminary experiments using AE15 at 0.1% (w/v).

Two methods were used to determine the uptake of ^{14}C into the plant. Radioactive surface deposits were removed by cellulose acetate stripping from the treated area of the leaf.[7] This method removes any superficial deposits together with the crystalline epicuticular

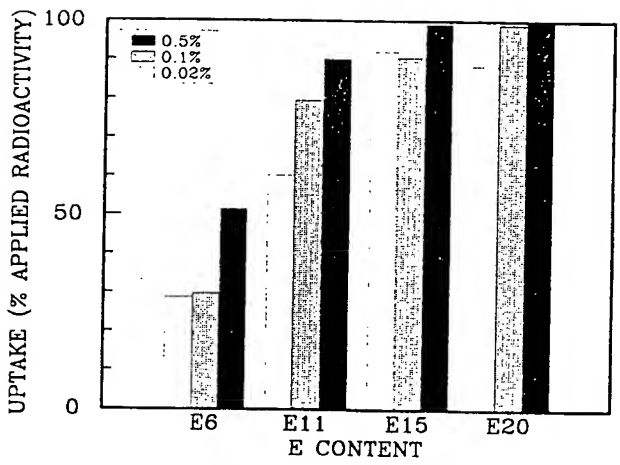

FIGURE 1. Influence of concentration and E content of a C_{13}/C_{14} alcohol series of surfactants on uptake of radiolabel from a 0.05% (w/v) solution of ^{14}C-methylglucose 1 d after droplet application to wheat leaves. In the absence of added surfactant, there was about 10% uptake of radiolabel. Values plotted are the means of four replicates; the standard error of the difference of the means for the 12 treatments is 4.5.

wax layer. Radioactivity was determined by liquid scintillation counter (LSC) analysis after the cellulose acetate strip was dissolved in a dioxan-based cocktail.

Combustion-LSC analysis of the excised treated area, after removal of the surface deposit, was used to determine the amount of radioactivity in the treated leaves. Tissue was oxidized in a stream of oxygen at 900°C and the $^{14}CO_2$ generated was trapped in an amine-based cocktail for subsequent LSC analysis. If the total amounts of radioactivity recovered from the surface deposit and within the treated area were substantially less than that originally applied, the proximal (basipetal movement) and distal portions (acropetal movement) of the treated leaf were combusted to quantify any movement of radioactivity.

III. RESULTS AND DISCUSSION

A. INFLUENCE OF SURFACTANT CONCENTRATION ON FOLIAR UPTAKE

There was a strong influence of surfactant concentration on the foliar uptake of the two test compounds (Figures 1 through 4), the highest surfactant concentration invariably promoting the greatest enhancement of uptake irrespective of surfactant structure. This is well illustrated by the uptake of radiolabel from ^{14}C-phenylurea on field bean after 1 d (Figure 4), where the uptake was about 20% in the presence of 0.02% (w/v) surfactant while it was about 95% at 0.5%. The highest surfactant concentration used often caused phytotoxic damage at the site of original droplet application; similar damage has been noted by workers with other surfactants.[5,6,8,14] However, in our experiments, the occurrence of such symptoms did not appear to impede foliar absorption.

The uptake data obtained also indicated that a threshold concentration of surfactant is probably necessary before significant activation of the uptake of any compound can occur. For example, for the uptake of ^{14}C-methylglucose into CE-grown wheat (Figure 1), the activation threshold of AE15 and AE20 is 0.02% or lower. However, a concentration in excess of 0.1% is required to activate a similar amount of uptake of phenylurea into the same species (Figure 2). The factors which govern these different threshold levels are not fully understood, but are likely to be the nature of the penetrating compound, the nature of

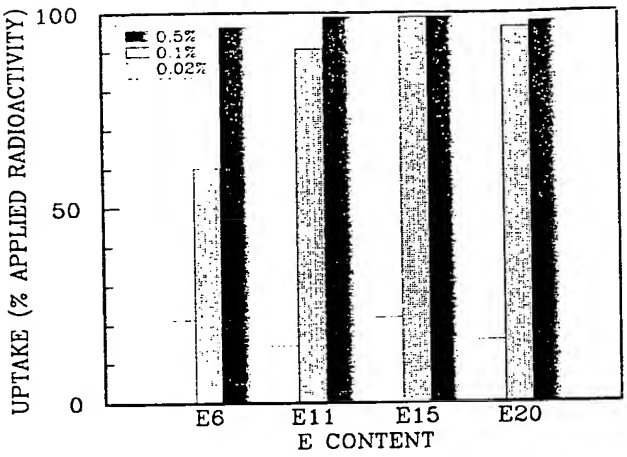

FIGURE 2. Influence of concentration and E content of a C_{12}/C_{14} alcohol series of surfactants on uptake of radiolabel from a 0.05% (w/v) solution of ^{14}C-phenylurea 1 d after droplet application to wheat leaves. In the absence of added surfactants, there was about 6% uptake of radiolabel. Values plotted are the means of four replicates; the standard error of the difference of the means for the 12 treatments is 4.3.

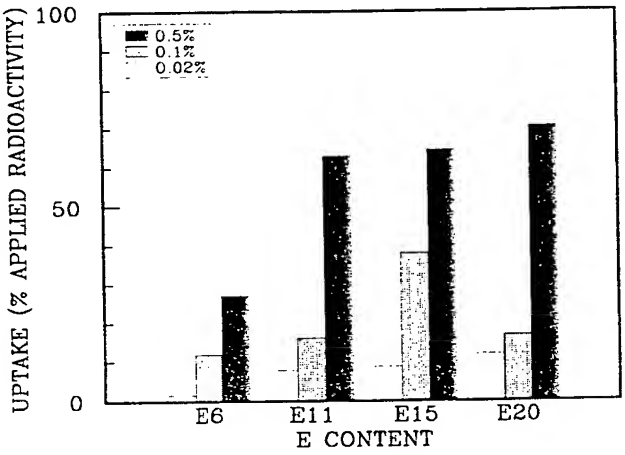

FIGURE 3. Influence of concentration and E content of a C_{13}/C_{14} alcohol series of surfactants on uptake of radiolabel from a 0.05% (w/v) solution of ^{14}C-methylglucose 1 d after droplet application to field bean leaves. In the absence of added surfactant, there was about 8% uptake of radiolabel. Values plotted are the means of four replicates; the standard error of the difference of the means for the 12 treatments is 5.2.

the surfactant, and the plant species treated.[4] It is planned to use radiolabeled surfactants added to formulations to investigate this phenomenon.

B. INFLUENCE OF SURFACTANT STRUCTURE ON FOLIAR UPTAKE

Surfactant structure had a significant influence on the foliar uptake of ^{14}C-methylglucose and ^{14}C-phenylurea on both plant species after 1 d (Figures 1 through 4). Similar effects have been observed for alkylphenol surfactants on the biological activity of three water-

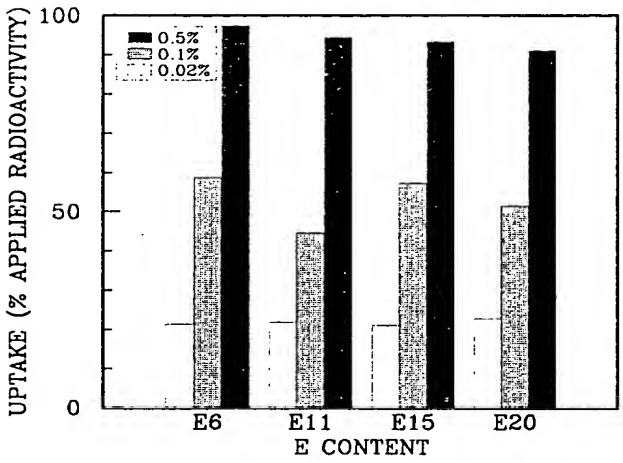

FIGURE 4. Influence of concentration and E content of a C_{13}/C_{14} alcohol series of surfactants on uptake of radiolabel from a 0.05% (w/v) solution of ^{14}C-phenylurea 1 d after droplet application to field bean leaves. In the absence of added surfactant, there was about 13% uptake of radiolabel. Values plotted are the means of four replicates; the standard error of the difference of the means for the 12 treatments is 5.2.

soluble herbicides on maize,[1,10] where there was an optimum E range of 15 to 20; higher and lower E members of the surfactant series were less effective activators. Such investigations, however, involved spray application with the complicating factors of retention and redistribution of the active ingredients. These factors are not present in the uptake work reported here.

Uptake activation of the highly water-soluble methylglucose is especially sensitive to E content, AE20 promoting the greatest uptake activation after 1 d (on wheat, 99.6% uptake of radiolabel in the presence of AE20 at 0.5%). Uptake of a related compound, 2-deoxyglucose, has been positively correlated with the hygroscopicity of octylphenoxy surfactants,[12] which increases with E content.[13] A moisture-retaining humectant action of AE surfactants, increasing with E content, is therefore a possible explanation for the activation results observed for methylglucose. This hypothesis could be confirmed by using ^{14}C-surfactants: it would be predicted that humectants will not penetrate, or penetrate at very low rates relative to that of the test compound, if they are to maintain the surface deposit of a soluble test compound in a hydrated state suitable for foliar uptake.

A relationship between the optimum foliar uptake of a compound, its log P, and the E content of alcohol polyoxyethylene surfactants has recently been proposed by us.[4] Using a response surface model, we postulate that there would be a shift in E content for optimal uptake-promoting properties from high E-content AE surfactants for low log P compounds to low E-content members for high log P compounds. However, in the central region of the model surface, the structural requirements in terms of E content for uptake promotion of compounds of intermediate log P are less distinct. Phenylurea is thought to be close to this so-called critical log P region, which is likely to vary according to plant species, thus explaining the relative lack of surfactant structural effects on the uptake of phenylurea, especially on field bean (Figure 4). A slight, but statistically significant, structural preference for higher E-content surfactants for uptake activation of phenylurea is, however, observed on wheat (Figure 2).

The findings reported here emphasize the need to select the correct surfactant structure and concentration for optimal activation of the foliar uptake of a particular compound.

TABLE 1
Uptake and Movement of Radiolabel in Field Bean 1 Day after Foliar Application of ^{14}C-Phenylurea Solutions (0.05%, w/v) Containing AE6 and AE15, Each at Three Different Concentrations

Surfactant	Concentration (%, w/v)	Surface[a] deposit	Region within treated leaf[b]		Recovery
			Treated area	Distal	
AE6	0.02	79 (5)	10 (1)	4 (1)	93
	0.1	41 (12)	47 (9)	13 (6)	101
	0.5	3 (1)	67 (10)	30 (10)	100
AE15	0.02	79 (10)	13 (4)	4 (1)	96
	0.1	43 (13)	44 (9)	15 (5)	102
	0.5	7 (2)	73 (3)	24 (3)	104

Note: Values expressed as a percentage of the radioactive dose applied; standard deviations of the mean of four replicates are given in parentheses.

[a] Determined by cellulose acetate film stripping.
[b] Determined by combustion analysis of cellulose acetate stripped leaves.

However, increasing the concentration of a less efficient activator surfactant may compensate for its lack of structural suitability. This is well illustrated by comparing the activating effects of AE6 at 0.5% and AE11 at 0.02% on the uptake of ^{14}C-methylglucose on wheat after 1 d (Figure 1). In this situation, a 25-fold greater concentration of AE6 is required to promote a magnitude of uptake activation similar to that of AE11 at 0.02%, i.e., 50% uptake of radiolabel compared to 60% for AE11.

Our findings also show that different uptake activation profiles for the same compound may be obtained, depending on the plant species treated. A good example is the uptake of ^{14}C-methylglucose on field bean (Figure 3) and on wheat (Figure 1), where a lower surfactant threshold is observed for wheat (0.02%), as is a more distinct gradient in the efficiency of uptake activation from AE6, which is least effective, to AE20, which promotes the greatest activation relative to the control treatment on both species. Superimposing data plotted in the form of response surfaces should reveal the maximum selective difference in the uptake of a compound between a target and nontarget species. This may not necessarily occur with the surfactant-concentration combination which produces the most efficient uptake activation on the target plant, such as a weed species. As Jansen et al.[5] have pointed out, judicious selection of surfactants could, therefore, lead to more effective targeting of pesticides.

C. INFLUENCE OF SURFACTANTS ON MOVEMENT OF RADIOACTIVITY

No movement of radiolabel out of the treated area was recorded for any formulations of ^{14}C-methylglucose applied to field bean leaves. Proximal and distal leaf portions were, accordingly, not retained for the subsequent experiment using ^{14}C-methylglucose on wheat. However, significant loss of radiolabel (about 20% of the applied dose) was observed following uptake of the same formulations by wheat. Subsequent experiments have revealed that this is due to a combination of acropetal transport and metabolism.

Proximal and distal portions of treated leaves were analyzed for AE6 and AE15 treatments in combination with ^{14}C-phenylurea on both wheat and bean plants. However, only acropetal transport of radiolabel was observed in both species. In field bean (Table 1), there would appear to be a general relationship between the amount of uptake and the quantity of radiolabel moving into the distal leaf portion 1 d after application. The amount of radiolabel in the

TABLE 2
Uptake and Movement of Radiolabel in Wheat 1 Day after Foliar Application of ^{14}C-Phenylurea Solutions (0.05%, w/v) Containing AE6 and AE15, Each at Three Different Concentrations

Surfactant	Concentration (%, w/v)	Surface deposit[a]	Region within treated leaf[b]		Recovery
			Treated area	Distal	
AE6	0.02	79 (6)	3 (1)	13 (1)	95
	0.1	40 (8)	8 (3)	49 (6)	97
	0.5	4 (1)	3 (1)	90 (2)	97
AE15	0.02	78 (8)	3 (1)	16 (3)	97
	0.1	1 (1)	4 (1)	91 (4)	96
	0.5	1 (1)	5 (2)	95 (4)	101

[a] Determined by cellulose acetate film stripping.
[b] Determined by combustion analysis of cellulose acetate stripped leaves.

distal leaf portion is about 25% of that recovered from within the treated leaf. Thus, there is no specific effect of surfactant structure or concentration on the movement of radiolabel. Results obtained from applications of ^{14}C-phenylurea to wheat illustrate a different pattern of movement of radioactivity (Table 2). Between 3 and 8% of penetrated radiolabel is retained within the treated area, while the bulk of the radioactivity is translocated into the distal leaf portion. Rapid movement in the acropetal direction therefore occurs following penetration. As with field bean, no specific relationship exists between movement of radiolabel and surfactant characteristics. The phytotoxic damage to the application site observed for AE15 at 0.5% had little influence on the transport of radioactivity in the treated leaves after 1 d.

It would thus seem probable that transport phenomena which occur after cuticular penetration of an agrichemical are not greatly influenced by surfactants — a suggestion first put forward by Foy and Smith.[1] However, in some situations, cellular damage may impair the movement of systemic compounds. Transport is determined, therefore, primarily by an interaction between the plant species and the test compound, while AE surfactants exert their influence almost exclusively at the penetration stage. Movement of nonionic surfactants away from the site of uptake is usually limited,[1,3,9] suggesting that their interaction with mobile active ingredients subsequent to uptake is minimal.

IV. CONCLUSION

The work conducted at LARS and elsewhere has shown that many problems remain unresolved in the field of foliar uptake, especially the exact mechanisms of uptake activation and the way in which such mechanisms may vary between different classes of surfactant. It is only from systematic uptake investigations of the type reported here, coupled with similar research on retention and distribution phenomena, that rational guidelines for optimal surfactant selection will be established. This should allow a predictive element in formulation development, ultimately reducing the amount of empirical work involved in the optimization of formulations. In addition, a better understanding of the mechanisms involved may lead to new uses for existing environmentally safe pesticides.

ACKNOWLEDGMENTS

The authors thank R. F. Hughes and his staff for raising plant material and maintenance of CE facilities, C. J. Marshall for assistance with computing and results analysis, and Mrs. J. Hynam for preparing the manuscript.

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Chapter 14

EFFECT OF POLYSORBATE SURFACTANTS WITH VARIOUS HYDROPHILIC-LIPOPHILIC BALANCE (HLB) VALUES ON LEAF SURFACE ULTRASTRUCTURE AND MOBILITY OF METHAZOLE IN PLANTS AND SOIL

Chester L. Foy and Tsuneyuki Takeno

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ABSTRACT

Polysorbate surfactant (hydrophile-lipophile balance [HLB] 4.3) eroded cotton (*Gossypium hirsutum* L.) leaf surfaces severely at 1% (w/w) concentration, as shown by scanning electron photomicrography. Reticulated and etched patterns were observed on cotton leaf surfaces treated with water-soluble surfactants. Trichomes on the leaves of prickly sida (*Sida spinosa* L.) did not appear to be affected by the surfactants. Leaves of prickly sida were less affected than those of cotton. The surface deposits of formulated methazole were different in appearance from those of technical methazole. The ^{14}C-methazole and/or its ^{14}C-labeled metabolites moved acropetally in the treated leaves of cotton and prickly sida. This pattern was not altered with polysorbate surfactants with different HLB values. Total uptake and distribution of ^{14}C increased with increasing concentration of methazole and decreasing HLB values of surfactants. More ^{14}C was translocated in cotton than in prickly sida. The effects of surfactants were masked by the drastic solvent action of 100% methanol. When the solvent effect was subtracted, the surfactant with HLB 8 caused the greatest enhancement of translocation in both species. Radiolabeled methazole leached poorly in a silt loam soil and was either slightly retarded or unaffected by added surfactants. The influence of HLB of polysorbate surfactants on ^{14}C-methazole mobility was minimal.

I. INTRODUCTION

Herbicides have been used to control weeds, one of the major economic problems in agriculture, for many years, and during this period many new herbicides with diverse chemical properties have been developed. Herbicides and plant growth regulators sprayed onto plant surfaces commonly encounter plant epidermal waxes as the first barrier to penetration. Multiple forms of wax excretions from leaves, shoots, and fruits were described by Amelunxen et al.[1] Measurement of the contact angle of a droplet applied to foliage is one method of determining the wettability of the plant surface by the solution. Hall et al.[9] combined contact angle determinations with scanning electron microscopy and found that contact angles in excess of 145° occurred on leaves when numerous wax rods or plates covered their surfaces. Silva Fernandes[24] studied the water repellency of surface waxes with electron microscopy and classified the surface waxes as water repellent and non-water repellent. A crystalline or semicrystalline wax repelled water and a noncrystalline wax did not repel water.

Wortman[31] used electron microscopy to study the changes in the surfaces of leaves caused by pesticides and a wetting agent. Ong et al.[23] observed leaf surfaces upon which herbicide was sprayed by using a scanning electron microscope (SEM) equipped to detect fluorescence and reported a new, rapid method for spatially localizing herbicides on leaf surfaces. Hess et al., using similar techniques, conducted a series of experiments on herbicidal dispersal patterns as a function of leaf surface,[10] mapping residues using X-ray fluorescence,[11] and as a function of formulation.[12]

Polysorbate surfactants, nonionic polyoxyethylated derivates of sorbitan fatty acid esters, have been used as additives to herbicidal formulations and are sometimes found to effectively enhance their herbicidal activity. The properties of nonionic surfactants are known to be changed with the hydrophilic-lipophilic-balance (HLB).

The optimum surfactant HLB requirement will change from system to system. Umoessien et al.[29] reported that the absorption and general phytotoxicity of linuron [*N'*-(3,4-dichlorophenyl)-*N*-methoxy-*N*-methylurea] and prometryn [*N,N'*-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine] were enhanced by surfactants within the HLB range of 5.4 to 15.0. Morton and Combs[21] found the greatest herbicidal enhancement of a picloram (4-

amino-3,5,6-trichloro-2-pyridinecarboxylic acid)-2,4,5-T [(2,4,5-trichlorophenoxy)acetic acid] mixture with surfactants within the HLB range of 13.3 to 15.4. Hull and Shellhorn[14] treated seedlings of mesquite (*Prosopis julifora* (Sw.) DC with the butoxyethyl ester of 2,4,5-T in various combinations of phytotoxic and nontoxic oils and nonionic surfactants of HLB 8.6 and 1.8, and found that when sorbitan monolaurate (HLB 8.6) was used with phytotoxic oils, subsequent apical epinasty and repression of growth was greater than with other combinations. Ethoxylated stearyl ether and amine surfactants at HLB 15 to 16 and 19 to 20, respectively, gave optimum effectiveness of glyphosate [*N*-(phosphonomethyl)glycine] against common milkweed (*Asclepias syriaca* L.) and hemp dogbane (*Apocynum cannabinum* L.); surfactants with a low HLB value were usually less effective.[32] Penetration of 2,4-D[(2,4-dichlorophenoxy)acetic acid] and NAA (2-naphthaleneacetic acid) in apple (*Malus pumila* M.) increased linearly as various surfactant HLBs decreased from 14.6 to 3.5.[27]

Tween 20 (HLB 16.7) showed a contact angle of 85.5 to 98.0% on intact leaf surfaces on four rice cultivars; these were the smallest in a Tween series having HLB values in the range of 11.0 to 16.7.[20] The surface tension of polyoxyethylene (POE) nonylphenyl ethers, POE octylphenyl ethers, POE sorbitans, and polystearylated phenols was lowest in the range of 12 to 14 HLB.[8] The contact angle on the leaf surface of rice (*Oryza sativa* L.) increased with an increase in HLB value, showing the lowest in the range of 10 to 13 HLB.

Chemical additives applied to plant or soil before, with, or after herbicide treatment reach the soil and, conceivably, may modify adsorption, absorption, mobility, leaching, diffusion, accumulation, and metabolism of the herbicide in such a way as to alter its activity and residual fate. Numerous studies involving the effects of adjuvants on plant surfaces[15] and in plants[22] have been conducted; however, relatively little information is available concerning the modifying effects of adjuvants on the distribution, availability, and persistence of herbicides in soil.[3]

Methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] is a selective herbicide for use in cotton (*Gossypium hirsutum* L.) and shows good selectivity in several other crops.[30] Methazole controls selected broadleaf weed species, including prickly sida (*Sida spinosa* L.). The extent and scientific bases of herbicidal selectivity between cotton and prickly sida have been reported.[6,7,16,17]

Bond and Roberts[4] reported little movement of methazole in soil in field studies. In leaching-column experiments, both methazole and its metabolites were relatively immobile.[5,26] The results of studies by Koskinen[19] indicated that methazole is highly adsorbed by soil organic matter, does not readily desorb, and can degrade rapidly.

The experiments described herein were conducted to evaluate the possible influence of polysorbate surfactants having different HLB values on (1) the plant cuticular structure and how polysorbate surfactants were deposited on the leaf surface, (2) absorption and translocation of ^{14}C-methazole in cotton and prickly sida plants (absorption and translocation of ^{14}C-labeled polysorbate surfactants were evaluated also), and (3) the depth of leaching of ^{14}C-methazole in soil.

II. MATERIALS AND METHODS

A. LEAF SURFACE STUDIES

Span 80® (nonionic surfactant containing sorbitan monooleate, HLB 4.3), Tween 80® (nonionic surfactant containing polyoxyethylene sorbitan monooleate, HLB 15.0), and their mixtures of 65/35 (w/w) Span 80/Tween 80 (HLB 8.0) and 28/72 (w/w) Span 80/Tween 80 (HLB 12.0) were used in this investigation. Surfactant solutions were prepared by first dissolving the surfactants in 5 g of absolute ethanol and then diluting to 100 g with distilled water. Also, an ethanol-aqueous solution (5%, w/w) and absolute ethanol were included to evaluate the solvent effect.

Methazole (99.4%, w/w) active solution was prepared by dissolving 0.8 g in 99.2 g of ethanol. Methazole in a 75% wettable powder form was prepared by adding 98.9 g of distilled water to 1.1 g of powder.

Cotton ("Deltapine") and prickly sida seedlings 8 to 10 and 18 to 20 d old, respectively, were placed in jars containing one-half strength Hoagland and Arnon's[13] nutrient solution No. 1 supplemented with 5 ppm Sequestrene 138 Fe [sodium ferric ethylenediamine di-(*o*-hydroxyphenyl acetate)]. The plants were grown under greenhouse conditions until the cotton and prickly sida plants were 30 and 45 d old, respectively. At the time of treatment, the cotton plants were 18 to 22 cm in height with two primary leaves fully expanded; prickly sida plants were 15 to 20 cm in height at the seven- to eight-leaf stage of growth.

The primary leaf of cotton and the fourth leaf of prickly sida were dipped for 1 min in 100 ml of 0.1 or 1.0% (w/w) polysorbate surfactant solution, or solvent, solvent-aqueous, or methazole spray solution. The treated leaves were cut at the petiole 3 and 72 h after treatment and two discs of approximately 6 mm in diameter were cut from each species. The leaf discs, one for study of the adaxial surface and another for observation of the abaxial surface, were fixed on metal holders with silver enamel and placed in a vacuum chamber. A uniform coating of palladium-gold was evaporated onto the surface. The samples were fully prepared within the day of harvest and observed by use of a high-resolution AMR Model 900 SEM.

B. MOBILITY STUDIES IN PLANTS

Stock solutions of Span 80 (HLB 4.3) and Tween 80 (HLB 15.0) were prepared by dissolving 200 mg of each in methanol and distilled water, respectively. Solutions with HLBs of 8 and 12.0 were prepared by mixing the stock solutions of the two surfactants at the same ratios described earlier.

The ^{14}C-methazole used was heterocyclic ring-labeled in the number 3 carbon (specific activity, 7.6 mCi/mmol). A stock solution was prepared by dissolving 100 μCi of ^{14}C-methazole in 10 ml of methanol. Various treatment solutions were made by dilution of the stock solution with surfactant solution, methanol, or distilled water.

In studies using radiolabeled surfactants, stock solutions of ^{14}C-Span 80 labeled in the fatty acid moiety (1 μCi/mg) and ^{14}C-Tween 80 labeled in the fatty acid moiety (0.25 μCi/mg) were prepared by dissolving 100 mg each in 10 ml of methanol and distilled water, respectively. Radiolabeled surfactant solutions with HLBs of 8.0 and 12.0 were prepared in the same manner as were nonlabeled surfactant solutions. Because of the different specific activities of Span 80 and Tween 80, the amount of radioactivity applied for each HLB value varied; therefore, count data were corrected for 0.1-μCi applications.

Cotton and prickly sida plants were grown and treated at the same ages and growth stages described earlier. A 20- or 25-μl drop containing ^{14}C-methazole (0.1 μCi) in methanol or 1% (w/v) surfactant solutions having different HLB values and methanol content was applied to the midrib of one primary leaf of cotton and the fourth leaf of prickly sida. In the treatments for studying foliar absorption of labeled surfactants, approximately 0.1 μCi of labeled surfactant in methanol or methanol-aqueous solution was applied in the same way. The treatment was localized by confining the solution in lanolin rings. Duplicate plants were harvested 3, 24, and 72 h after treatment. At the end of the treatment periods, the lanolin paste was removed and the treated spot was removed with a cork borer. The plants were sectioned into roots, stems, and leaves and processed for autoradiography. Following autoradiography, treated lamina and their petioles were ground, suspended in Aquasol Universal L.S.C. Cocktail, and counted using a 3000 Series Packard Tri-Carb liquid scintillation counter.

C. MOBILITY STUDIES IN SOIL

A stock solution was prepared by dissolving 100 μCi of ^{14}C-methazole in 10 ml of acetone. Various treatment solutions were made by diluting the stock solution with nonradiolabeled methazole, surfactant, and acetone. The same surfactants — Span 80, Tween 80, and their mixtures — employed in earlier experiments were used.

Plastic straws 6 mm in diameter and 21 cm long were used as soil columns. The bottom of each column was packed with a small amount of glass wool. The columns were uniformly filled with 4.5 g of a silt loam soil (1.4% organic matter) which had been oven dried at 70°C for 10 d and sieved to pass through a 40-mesh screen.

Methazole at 0.22 g/m^2 and surfactants at these concentrations were applied in a 20-μl acetone solution to the surface of the soil so that there was 0.25, 1.0, and 4.0 times as much surfactant present on a weight basis. Each 20-μl treatment solution contained 1.9 μg (0.1 μCi) of ^{14}C-methazole and 3.1 μg of nonradiolabeled methazole.

After the soil had dried from the herbicide treatment, the columns were leached with 10 ml of distilled water, which was added continuously to maintain a water level at least 5 mm deep on the soil surface. The soil columns were kept at the same position for 4 h after irrigation and then placed at the horizontal position in a 70°C oven for 24 h. The columns were then sectioned in 1-cm lengths from the top of the soil and oven dried an additional 48 h. The straw shells were removed and the soil was suspended in a liquid scintillation cocktail and counted as described earlier.

III. RESULTS AND DISCUSSION

A. LEAF SURFACE STUDIES

SEM micrographs of natural leaf surfaces of cotton, which did not receive any treatment, are shown in Figures 1A and 1B. The cuticle in the cotton covered the epidermal cells smoothly, and it was difficult to locate the boundary between cells as reported by Troughton and Donaldson.[28] The stomata appeared to be somewhat more numerous on the abaxial than on the adaxial side of the leaves (Figures 1A through 1F). Both surfaces of the cotton treated with 100% ethanol were dehydrated and exhibited complex fibrous wax structures (Figures 1C and 1D). A louver-like structure of leaf waxes was observed outside the guard cells on the adaxial surface. These results suggest that the ethanol solubilized and removed some of the waxes from the leaf surfaces, so that they were dehydrated and shrunken. Figures 1E and 1F illustrate the results of the treatment of cotton with 5% (w/w) ethanol-aqueous solution, which was used as a solvent for polysorbate surfactants. The epidermal cells were shrunken and the surfaces wrinkled. The stomata of the abaxial surface were open wider than those of the adaxial surface (Figures 1E and 1F).

Leaf surfaces of cotton treated with 1% (w/w surfactant, HLB 4.3) solutions were more drastically affected than those treated with other surfactant solutions (Figures 2A through 2H). The surfactant accumulated in the troughs of irregular corrugations of the leaf surface 3 h after treatment (Figure 2A), and then severely eroded the leaf wax structure 72 h after treatment (Figure 2B). The stomatal openings were masked with Span 80 and/or eroded leaf waxes (Figure 2B). Accumulation of the surfactant in the troughs of irregular corrugations was also observed with 0.1% (w/w) surfactant (HLB 4.3), but severe erosion of the surface waxes was not observed (micrographs not shown). Erosion and etching of leaf surfaces were not observed with 1% (w/w) surfactant with HLB 8.0 (Figures 2C and 2D). Cotton leaves treated with 1% (w/w) surfactant (HLB 12.0) had a network of cracked cuticle which covered the epidermal cells 3 h after treatment (Figure 2E). The stomata of the leaf 72 h after treatment with the same solution had not closed at that stage, but other epidermal cells shrank and the surface became wrinkled (Figure 2F). Cotton leaves treated with 1.0% (w/

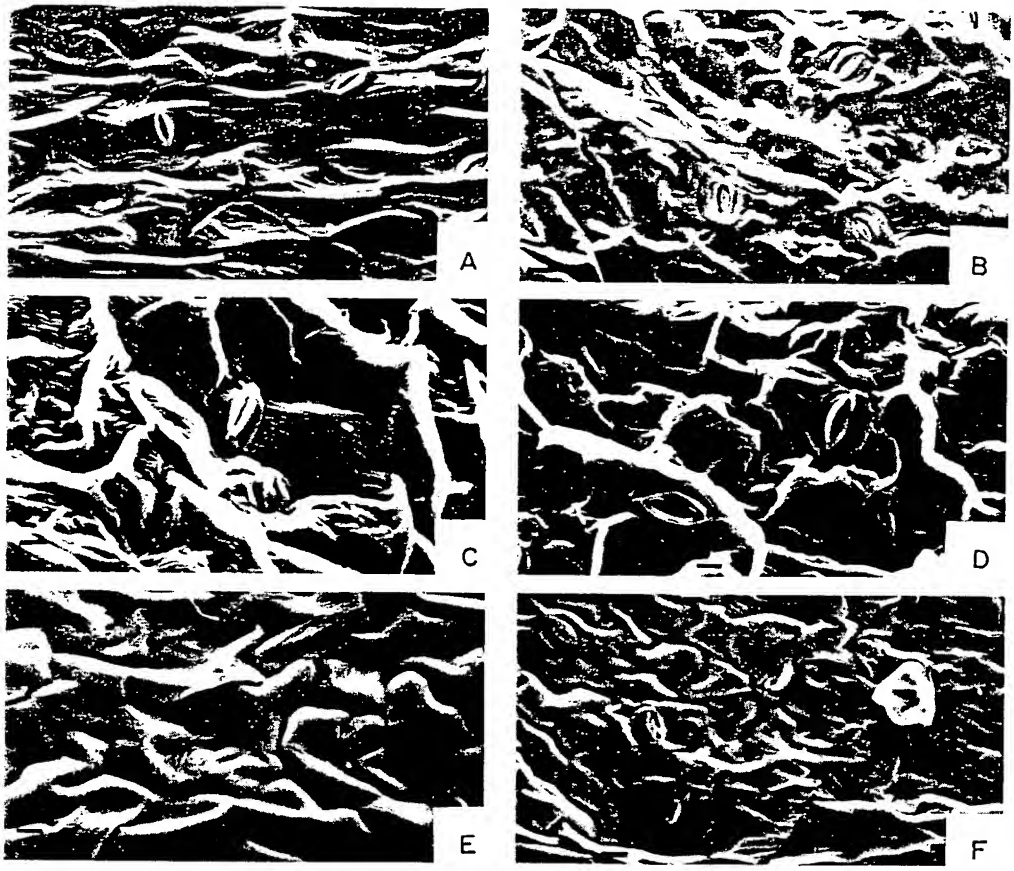

FIGURE 1. SEM micrographs of cotton leaf surfaces. (A) Adaxial surface of untreated leaf; (B) abaxial surface of the same leaf; (C) adaxial surface of leaf 3 h after dipping in 100% ethanol; (D) abaxial surface of the same leaf; (E) adaxial surface of leaf 3 h after dipping in 5% (w/w) ethanol-aqueous solution; (F) abaxial surface of the same leaf. Scale represents 10 μ.

w) surfactant (HLB 15.0) had cracked surfaces (Figure 2G and 2H) which resembled leaf surfaces treated with polysorbate surfactant with HLB 12.0. The pattern may have been water-soluble surfactants themselves, which spread over the leaf, dried, and then cracked because of low affinity for leaf waxes.

The leaf surfaces of prickly sida which did not receive any treatment are shown in Figures 3A and 3B. The leaf surfaces of prickly sida appear smooth to the naked eye, but numerous trichomes were observed on both sides of the leaf by SEM. These trichomes have a narrow, needle-like upper part and some of them have branched, multicellular, stellate hairs (Figures 3B and 3D). Prickly sida leaves dipped in 100% ethanol were severely dehydrated (Figures 3C and 3D). Leaf surfaces treated with 5% (w/w) ethanol solution were similar to those of untreated leaf, suggesting that the 5% ethanol solution did not significantly influence the cuticle of the prickly sida leaf (Figures 3E and 3F).

About 3 h after treatment with surfactant (HLB 4.3) solution, numerous small brown spots appeared on both sides of the leaf of prickly sida; they then turned black within 72 h. SEM micrographs taken over those discolored regions of the leaf show that Span 80 was

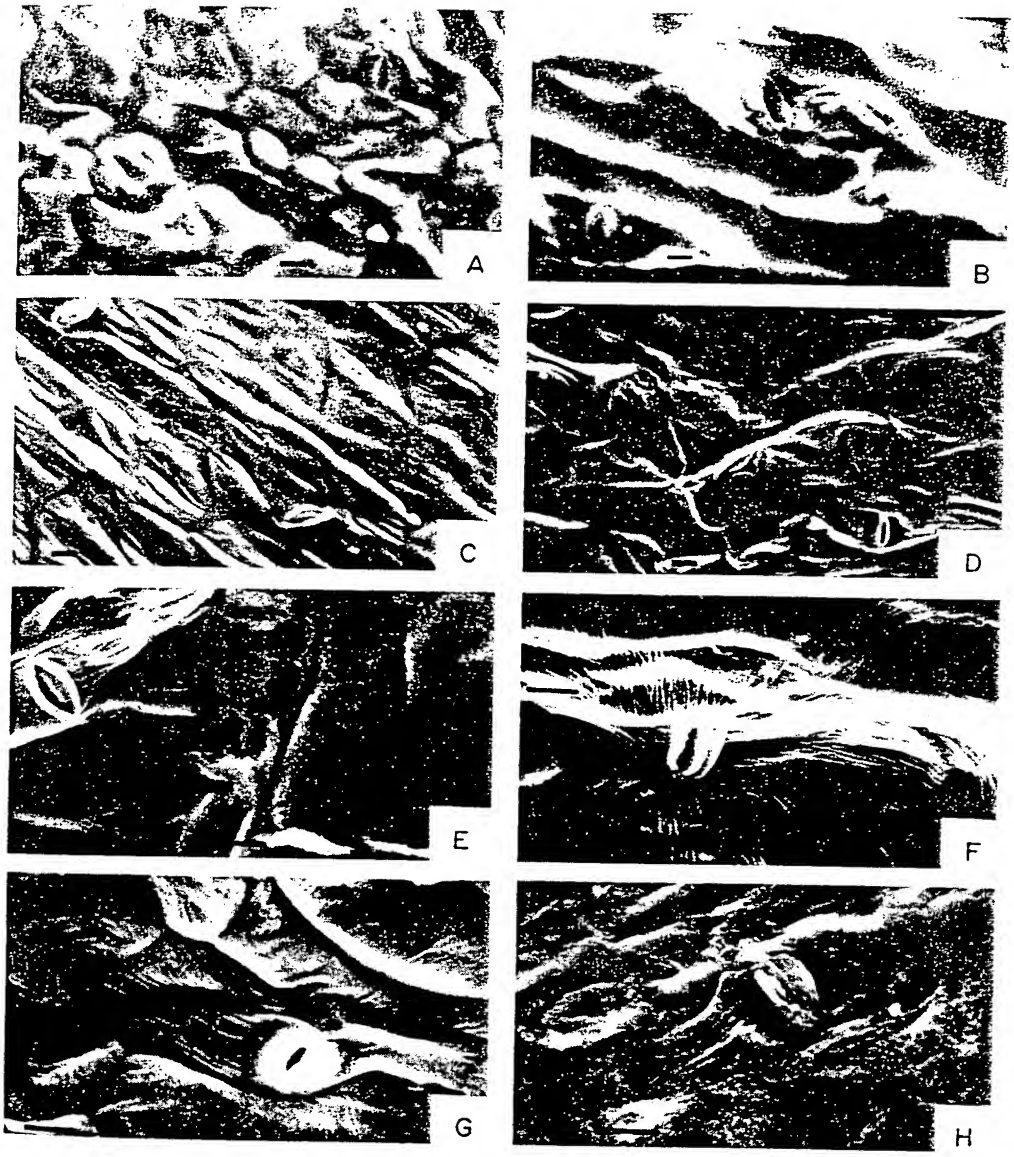

FIGURE 2. SEM micrographs of adaxial surfaces of cotton after dipping in 1% (w/w) surfactant solutions. HLB 4.3 at 3 h (A) and 72 h (B) after dipping; HLB 8.0 at 3 h (C) and 72 h (D) after dipping; HLB 12.0 at 3 h (E) and 72 h (F) after dipping; HLB 15.0 at 3 h (G) and 72 h (H) after dipping. Scale represents 10 μ.

deposited on the leaf surfaces like oil drops (Figures 4A and 4B). Prickly sida leaves treated with surfactant (HLB 8.0) solutions were more dehydrated than those treated with preparations with other HLB values (Figures 3A, 3B, and 3E through 3H). Figures 4E through 4F are SEM micrographs of prickly sida treated with water-soluble surfactant (HLB 12.0 and 15.0) solutions. No remarkable changes in leaf cuticle were observed. Trichomes were apparently not affected by these surfactants.

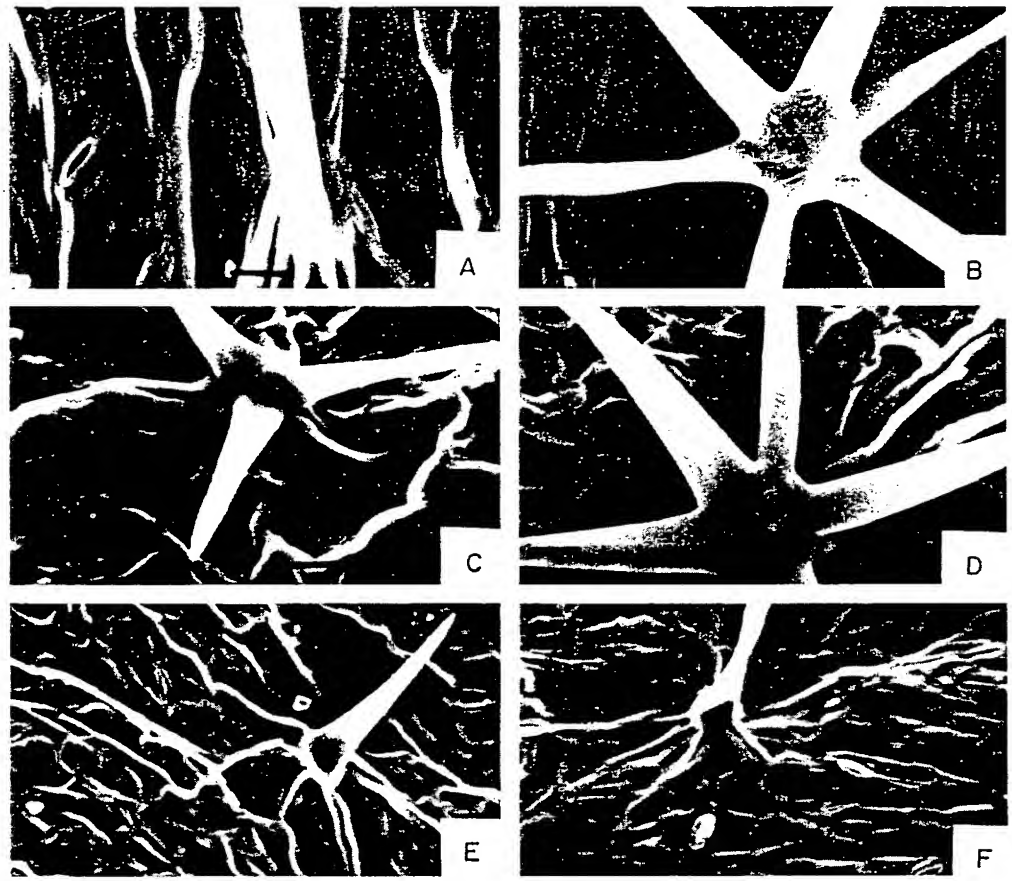

FIGURE 3. SEM micrographs of prickly sida leaf surfaces. (A) Adaxial surface of untreated leaf; (B) abaxial surface of the same leaf; (C) adaxial surface of leaf 3 h after dipping in 100% ethanol; (D) abaxial surface of the same leaf; (E) adaxial surface of leaf 3 h after dipping in 5% (w/w) ethanol-aqueous solution; (F) abaxial surface of the same leaf. Scale represents 10 μ.

Crystallized methazole was observed on the leaf surfaces of both plant species treated with 0.8% (w/w) technical methazole ethanol solution (Figures 5A and 5C). Wettable powder suspensions of methazole caused deposits on the leaf surfaces of both plant species that were readily observed (Figures 5B and 5D). The amounts of chemicals deposited on the leaves of prickly sida were greater than on the leaves of cotton.

B. MOBILITY STUDIES IN PLANTS

Autoradiograms of the 72-h foliar treatment of cotton and prickly sida indicated that methazole remained in the treated leaf and moved apoplastically, regardless of the surfactant HLB and methanol content (Figure 6). The distribution of ^{14}C in the veins and closely associated tissues of both cotton and prickly sida increased with the concentration of methanol in the treatment solutions, and also increased as the HLB of surfactants became lower or more liphophilic. Radioactivity in the laminas and petioles of treated leaves was determined for both plants. The pattern of accumulation of ^{14}C was similar in both plant tissues, but

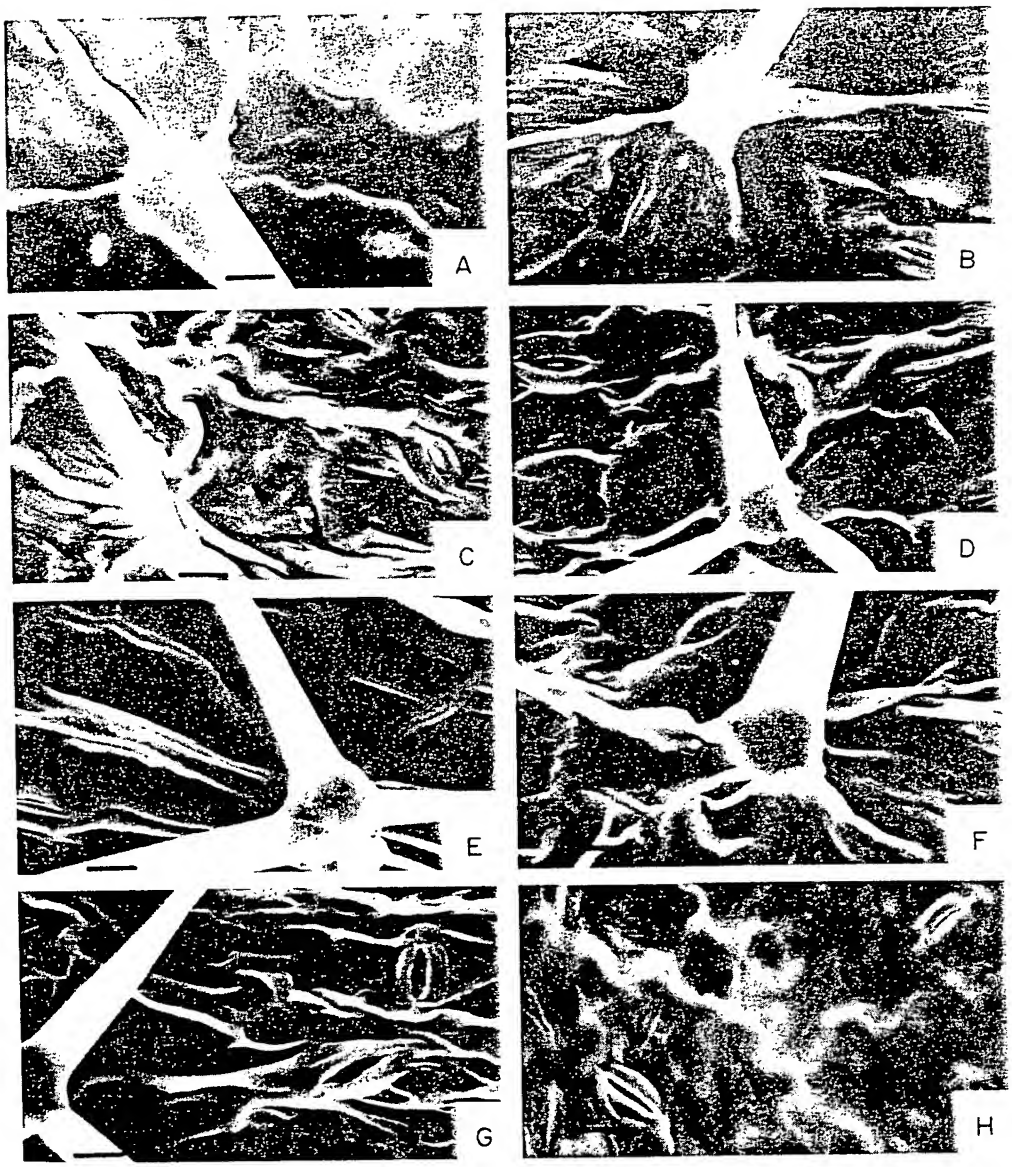

FIGURE 4. SEM micrographs of prickly sida leaf surfaces treated with 1% (w/w) surfactant solution. (A) Abaxial surface of leaf 3 h after dipping in surfactant (HLB 4.3) solution; (B) adaxial surface of leaf 72 h after dipping in the same solution; (C) adaxial surface of leaf 3 h after dipping in surfactant (HLB 8.0) solution; (D) abaxial surface of the same leaf; (E) adaxial surface of leaf 3 h after dipping in surfactant (HLB 12.0) solution; (F) adaxial surface 72 h after dipping in the same solution; (G) adaxial surface of leaf 72 h after dipping in surfactant (HLB 15.0) solution; (H) abaxial surface of the same leaf. Scale represents 10 μ.

the levels of radioactivity were much smaller in the petioles and significant differences were not observed among treatments. Therefore, only data for radioactivity in the laminas are presented (Tables 1 through 3). More ^{14}C-activity in cotton leaves was associated with solutions containing the surfactant blend with HLB 8.0 in 82.5% methanol at each time

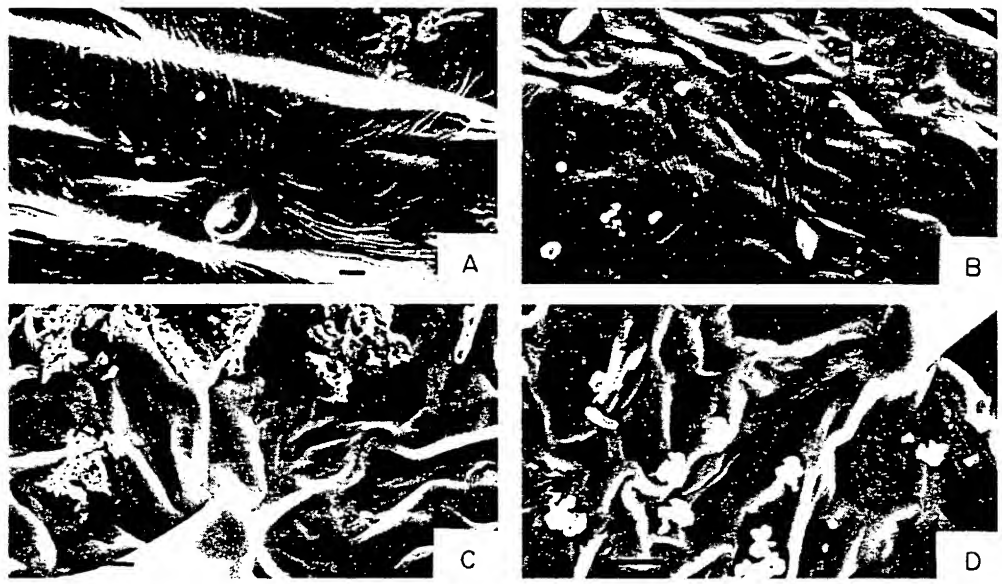

FIGURE 5. SEM micrographs of leaf surfaces treated with methazole. (A) Adaxial surface of cotton leaf 72 h after dipping in 0.8% (w/w) technical methazole-ethanol solution; (B) adaxial surface of cotton leaf 72 h after dipping in 1.0% (w/w) formulated methazole-distilled water solution; (C) adaxial surface of prickly sida leaf 72 h after dipping in 0.8% (w/w) technical methazole-ethanol solution; (D) adaxial surface of prickly sida leaf 72 after dipping in 1.0% (w/w) formulated methazole-distilled water solution.

after treatment (Table 1). As the treatment time increased, radioactivity in the treated leaf increased except in the case of the surfactant with HLB 15.0 in 50% (v/v) methanol. Those increases were not statistically significant, however.

There were more distinct differences in the distribution of ^{14}C in the treated leaf outside of the treated area among treatments in the case of prickly sida than in cotton despite less uptake in prickly sida (Table 1). Uptake of ^{14}C-methazole was maximal with the 1% surfactant (HLB 4.3) — 99% methanol solution at each treatment time. These observations suggest that the mobility of ^{14}C-methazole varies with plant species as well as the composition of the formulation.

In the previous experiment, the treatment solutions were prepared using methanol and aqueous-methanol solutions because of the low water solubility of ^{14}C-methazole and Span 80 (HLB 4.3). In this experiment, the concentrations of methanol were adjusted to the same levels as in the previous experiment (Table 1), to examine the effect of methanol concentration alone and to compare its effect with that of surfactants plus methanol or aqueous methanol. Absorption and translocation of ^{14}C increased slightly with increasing methanol concentration up to 82.5%, with the maximum effect at 100% methanol for cotton and prickly sida (Table 2). The difference in ^{14}C translocated when 100% methanol was employed, rather than other methanol concentrations of 40 to 82.5% (v/v), was remarkable for both species. These observations suggest that the addition of 17.5% (v/v) or more water to the treatment solution considerably reduces the effect of methanol on the uptake and translocation of ^{14}C-methazole in both species.

In a third experiment, ^{14}C-methazole was applied in solutions containing 1% surfactant and 99% methanol or in 100% methanol. The surfactants did not significantly increase the

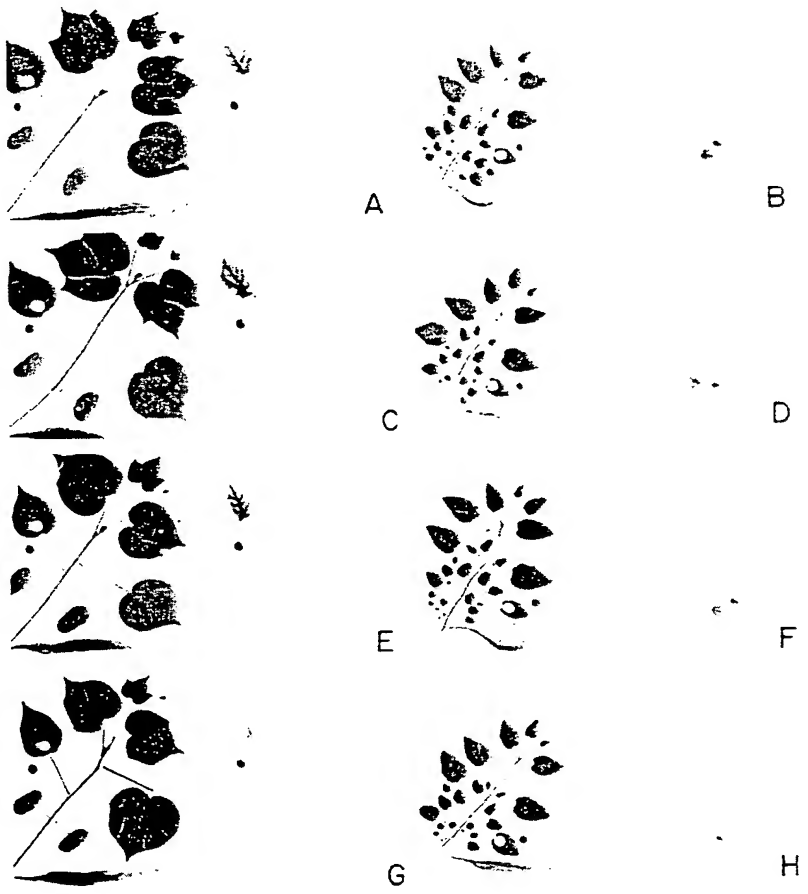

FIGURE 6. Accumulation of radioactivity after 72 h in cotton (left) and prickly sida (right) plants treated with 0.1 μCi of ^{14}C-methazole with 1% (w/v) polysorbate surfactant in methanol or methanol-aqueous solution. Plants left: autoradiograms right. HLB of surfactant used and methanol content (%, v/v) of treatment solutions, respectively: (A) and (B) 4.3, 99.0; (C) and (D) 8.0, 82.5; (E) and (F) 12.0, 64.0; (G) and (H) 15.0, 50.0.

accumulation of ^{14}C outside of the treated areas in leaves of cotton after 72 h (Table 3). The methanol used in this experiment masked the effect of polysorbate surfactants as well as the effect of the HLB of these surfactants, suggesting that the effects seen in Figure 1 and Table 1 were methanol effects rather than HLB effects. In fact, polysorbate surfactant tended to restrict the influence of the 100% methanol solution on ^{14}C-methazole uptake in prickly sida (Table 3).

Autoradiograms (not shown) and quantitative data (not shown) indicated no distribution of ^{14}C-fatty acid-labeled polysorbate surfactants in either species: no visible distribution of ^{14}C occurred with any methanol concentration, any HLB of surfactants, or at any treatment time. Smith and Foy[25] reported similar observations on the movement of labeled polysorbate surfactants.

Because of the effect of 100% methanol on absorption and translocation, the effects of polysorbate surfactants themselves as well as their HLB effect were nullified when they were used in 100% methanol. Therefore, it is considered inappropriate to judge the effect

TABLE 1
Accumulation of ^{14}C-Methazole Outside the Treated Areas in Primary Leaves of Cotton and Fourth Leaves of Prickly Sida Following Application in 1% Surfactant Solutions Containing Various Amounts of Methanol

Species	Time after treatment (h)	Surfactant HLB and methanol content (v/v)			
		4.3, 99%	8.0, 82.5%	12.0, 64%	15.0, 50%
		10^3 dpm			
Cotton	3	19.3 ab	21.8 ab	7.0 b	6.1 b
	24	28.9 ab	33.4 ab	17.8 ab	9.5 b
	72	34.6 a	39.6 a	22.5 ab	9.0 b
Prickly sida	3	2.4 cd	1.1 d	0.8 d	0.3 d
	24	6.3 ab	4.2 bc	2.0 cd	1.1 d
	72	8.2 a	6.5 ab	5.3 b	2.6 cd

Note: Means followed by the same letter within a species do not differ at the 5% level according to Duncan's multiple range test.

TABLE 2
Accumulation of ^{14}C-Methazole Outside the Treated Areas in Primary Leaves of Cotton and Fourth Leaves of Prickly Sida Following Application in Methanol or Aqueous Methanol

Species	Time after treatment (h)	Methanol content (%, v/v)				
		100.0	82.5	64.0	50.0	40.0
		10^3 dpm				
Cotton	3	20.2 c	5.8 fg	7.1 fg	6.0 fg	4.5 g
	24	37.6 a	11.7 e–g	12.5 ef	10.6 e–g	9.8 e–g
	72	31.4 b	19.5 cd	13.5 c–f	10.4 e–g	15.5 c–e
Prickly sida	3	4.0 c	0.6 g	0.4 g	0.3 g	0.4 g
	24	7.4 b	0.8 fg	0.6 fg	0.4 g	0.5 g
	72	12.3 a	2.3 d	1.8 d	1.0 de	1.5 ef

Note: Means followed by the same letter within a species do not differ at the 5% level according to Duncan's multiple range test.

of the HLB of the surfactants by subtracting the value for the distribution of ^{14}C with methanol alone from those for the distribution of ^{14}C with surfactant and methanol. Figure 7 shows the results, with both plant species, of subtracting the value for the distribution of ^{14}C with aqueous or absolute methanol from those for the distribution of ^{14}C with polysorbate surfactants in the same solution. The HLB effects of polysorbate surfactants were similar in both plant species. The effect of surfactant was greatest with HLB 8.0 on the translocation of ^{14}C-methazole in cotton plants. The plots below zero obtained at HLBs of 4.3 for both species are the result of the effect of 100% methanol being greater than 99% methanol plus surfactant. Also, the plots below zero obtained at HLB 15.0 for cotton are the result of 50% methanol being greater than 50% methanol plus surfactant. Thus, the method used (Figure 7) may not fully differentiate HLB effects. This method does, however, demonstrate consistent differences in the absorption and translocation of ^{14}C-methazole that were correlated with HLB.

TABLE 3
Accumulation of ^{14}C-Methazole Outside the Treated Areas in Primary Leaves of Cotton and Fourth Leaves of Prickly Sida Following Application in Solutions Containing 1% Surfactant and 99% Methanol or in 100% Methanol

Species	Time after treatment (h)	Surfactant HLB				
		4.3	8.0	12.0	15.0	Methanol
		10^3 dpm				
Cotton	3	19.3 a	14.0 b	12.9 b	12.1 b	20.2 a
	24	28.9 a	35.3 a	31.8 a	22.6 a	37.5 a
	72	34.6 a	43.5 a	39.5 a	44.9 a	31.4 a
Prickly sida	3	2.4 ef	3.7 f	1.8 f	1.6 f	4.0 e
	24	6.3 cd	4.3 de	6.5 cd	3.7 e	7.4 c
	72	8.2 bc	7.3 c	7.5 c	9.4 b	12.3 a

Note: Means followed by the same letter within a species do not differ at the 5% level according to Duncan's multiple range test.

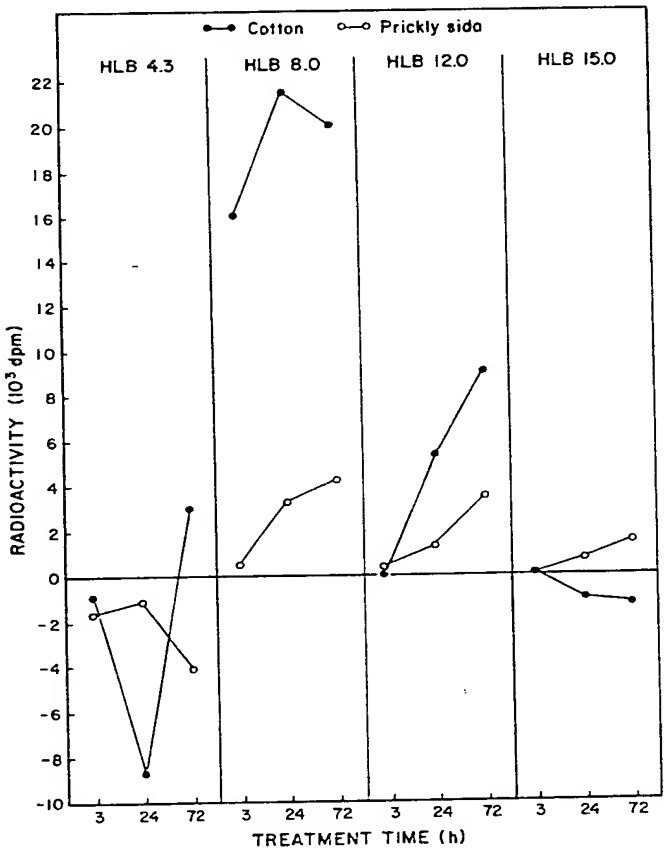

FIGURE 7. Influence of the HLB of polysorbate surfactants on absorption and translocation of ^{14}C-methazole in the primary leaf of cotton and the fourth leaf of prickly sida plants.

C. MOBILITY STUDIES IN SOIL

The degree to which soil adsorbed ^{14}C-methazole when polysorbate surfactants were present is shown in Figure 8. All treatments showed the greatest amount of residue of ^{14}C-methazole in the soil within the first 1 cm of depth. The amount of the residue decreased in proportion to the depth in the soil column, indicating that the herbicide remained predominantly in the top layers of the soil, as reported by other researchers.[4,5,19,26]

When methazole was applied with one fourth as much surfactant, approximately 85% of the total ^{14}C applied was detected in the soil within 4 cm of the surface regardless of the HLB of the polysorbate surfactant used. The degree of ^{14}C adsorption at the same soil depth decreased slightly with the increase of surfactant, while the radioactivity of the treatment with the ^{14}C-methazole without surfactant was approximately 76% at the same soil column depth. However, approximately 96% or more of the radioactive material was determined in the soil within 7 cm in depth for all treatments; at this level, not only the HLB of the polysorbate surfactant, but also the polysorbate surfactant itself did not show any influence on the leaching of heterocyclic ring-labeled ^{14}C-methazole.

When the ratio of the polysorbate surfactant and herbicide was the same, the influence of the HLB of the polysorbate surfactant was minimal. The results of the study on soil adsorption of substituted urea herbicides as influenced by surfactants showed that nonionic and anionic surfactants have either no effect or decrease the adsorption of these herbicides.[2] Bayer[2] reported that Tween 20 (nonionic surfactant containing polyoxyethylene sorbitan monolaurate, HLB 16.7) increased the leaching depth of diuron [*N*-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea] by increasing the concentration of the surfactant.

Our results suggest that adsorption of ^{14}C-methazole onto the soil surface was different from that of diuron. Why relatively low amounts of polysorbate surfactant restrict the movement of the herbicide more than greater amounts of the surfactant is not explained.

Koren et al.[18] reported that the movement of thiocarbamate herbicides was directly related to the water solubility of the herbicide and inversely related to the organic matter content of the soil. Our experimental results confirm this concept; the water solubility of methazole is 1.5 ppm at 25°C, and it was adsorbed on the soil in the top layers of the column. Tween 80 (HLB 15.0), a water-soluble surfactant, is used as a solubilizer in numerous industrial fields. However, even when methazole was applied with four times as much Tween 80, no significant difference in adsorption of ^{14}C was observed, compared to the addition of the same amount of less water-soluble polysorbate surfactant.

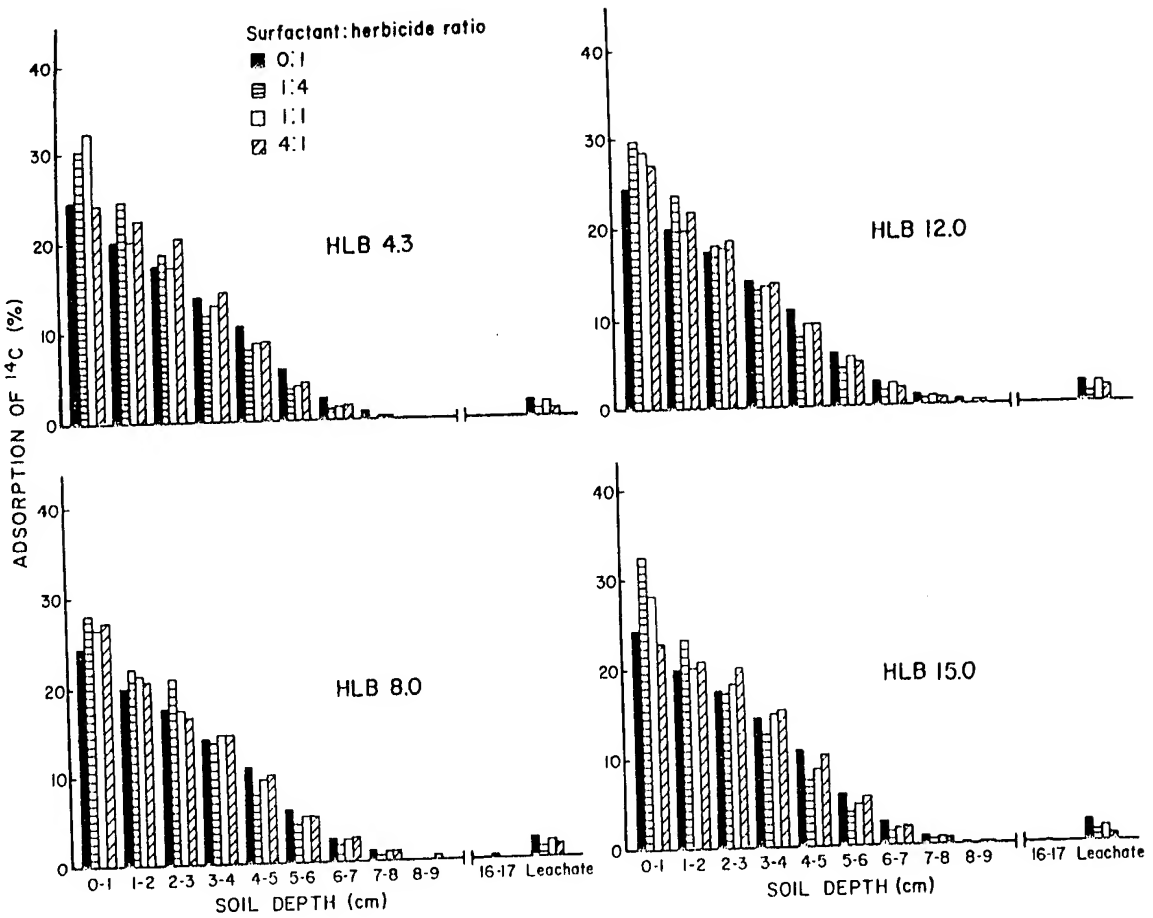

FIGURE 8. Influence of the amounts of surfactants with various HLB values on the leaching of ^{14}C-methazole, expressed as percent adsorption of ^{14}C-methazole on silt loam soil.

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Chapter 15

CHLOROPHYLL FLUORESCENCE — A NONINVASIVE TECHNIQUE FOR RAPID INVESTIGATION OF THE EFFECTS OF ADJUVANTS ON HERBICIDE AND PLANT GROWTH REGULATOR UPTAKE BY LEAVES

Mick P. Percival, Mark H. Blowers, John W. Green and Neil R. Baker

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ABSTRACT

Chlorophyll fluorescence measurements were used to investigate aspects of uptake by wheat leaves of the photosynthetically active herbicide diuron, the amino acid biosynthetic inhibitor herbicide glyphosate, and the plant growth regulator (PGR) chloromequat.

Quantitative changes in fluorescence, detected within 60 min of treatment, correlated directly with the concentration of diuron incorporated into leaf tissue. Subsequent fluorescence studies demonstrated that the formulation with Tween 20 enhanced diuron uptake. Further enhancement of diuron uptake by removal of the epicuticular wax layer was unaffected by Tween 20. Maximum uptake of diuron was achieved with Tween 20 concentrations near its critical micelle concentraction (cmc) value. Fluorescence measurements were also used for the rapid selection of the most effective formulations of the photosynthetic herbicides (phenmedipham and bentazon) with commercial adjuvants.

Chlorophyll fluorescence emitted from young wheat leaves showed perturbation 24 h after treatment with the nonphotosynthetic herbicide glyphosate and the PGR, chlormequat. Rapid selection of the most effective adjuvant formulations of these compounds was also demonstrated using fluorescence measurements.

The results demonstrate that chlorophyll fluorescence measurements can be used to investigate and optimize herbicide and PGR uptake by leaves as well as for the rapid screening of different adjuvant formulations of herbicides and PGRs.

I. INTRODUCTION

The use of adjuvants can improve the efficacy of herbicide and plant growth regulator (PGR) postemergent applications, often leading to significant gains both in the cost efficiency of the treatment and in diminished environmental impact. Yet, without quantitative *in vivo* probes of herbicide and PGR uptake, the effects of adjuvants are poorly understood and seldom fully optimized, since this often requires expensive and time-consuming field trials. It may be possible, however, to address many of these problems both rapidly and cost effectively using chlorophyll fluorescence measurements from treated leaves.

It is well known that chlorophyll fluorescence emission from photosynthetic tissues can be used as a noninvasive probe to monitor photochemical events *in vivo*.[2] For example, photochemical changes occurring in developing photosynthetic tissues and as a result of environmental stresses can be examined using fluorescence techniques.[1,8,9] Fluorescence analyses have also often been used to investigate the effects of herbicides on photosynthesis, particularly compounds which bind to the D1 protein of the photosystem 2 (PS2) reaction center.[7]

In this chapter, we demonstrate that chlorophyll fluorescence can be used to investigate aspects of herbicide uptake in leaves using the photosynthetically active herbicide, diuron [*N'*-(3,4-dichlorophenyl)-*N*,*N*-dimethylurea]. This technique enabled optimal adjuvant formulations of two other photosynthetically acting herbicides, phenmedipham {3-[(methoxycarbonyl)amino]phenyl(3-methylphenyl)carbamate} and bentazon [3-(1-methylethyl)-(1*H*)-benzo-thiadiazin-4(3*H*)-one-2,2-dioxide], to be selected. We also demonstrate how fluorescence techniques can be used to screen adjuvant formulations of the nonphotosynthetic herbicide, glyphosate[(*N*-phosphonomethyl)glycine], and the PGR, chlormequat (2-chloroethylmethyl-ammonium chloride).

II. MATERIALS AND METHODS

Wheat (*Triticum aestivum* cv. Broom) seedlings were grown in controlled environment cabinets at 20°C under a mean photosynthetically active photon flux density (ppfd) of 250

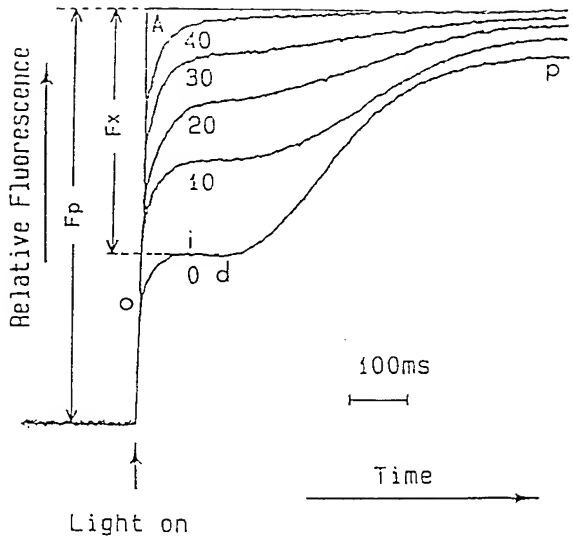

FIGURE 1. Changes in chlorophyll fluorescence induction curves of a wheat leaf after treatment with 0.045 m*M* diuron are quantified using Fx/Fp or area (A) over induction curve measurements.[6] Times after treatment are given in min.

μmol m^{-2} s^{-1} and a 16-h photoperiod at constant relative humidity (RH = 70%). Seven-day-old (single-leaf stage) seedlings, selected for uniformity, were used in investigations with the herbicides diuron, phenmedipham, and bentazon. Eleven-day-old (two-leaf stage) seedlings were used in the chlormequat and glyphosate treatments. All seedlings were treated by total immersion of aerial tissue for 5 s in the herbicide and PGR formulations were described. Chlorophyll fluorescence was monitored, by methods previously described, from a 1-cm wheat leaf segment 2 cm from the tip of 6-d-old leaves and from a 3-cm leaf section located 9 cm from the base of the youngest leaf in 11-d-old seedlings.[4,6] Radiolabeled diuron incorporation was determined by scintillation after treatment of 0.5 cm^2 of the 1-cm leaf segment of 6-d-old leaves with 25 μl of ^{14}C-diuron (2 m*M*), followed by extraction into 2 ml of ethanol after chlorophyll fluorescence had been measured. Epicuticular wax was removed from leaves by the application and subsequent stripping of a cellulose acetate (4%, w/v) film.

III. RESULTS AND DISCUSSION

Most chlorophyll fluorescence emission is derived from excited chlorophylls associated with PS2 complexes in chloroplast membranes. Upon illumination, dark-adapted leaves exhibit a rapid induction of fluorescence which primarily reflects initial photochemical events associated with PS2 (see curve for 0 min, Figure 1). The rapid fluorescence induction has been described by the nomenclature o, i, d, and p (Figure 1).[5] The minimal fluorescence level attained when PS2 is maximally oxidized in nonenergized membranes is represented by o. The i–d inflection and the level of fluorescence at Fi correlate with the redox state of the primary quinone electron acceptor (Q_A) in PS2. The rise to p and fluorescence level at Fp are governed by reduction of the plastoquinone (PQ) pool (secondary electron acceptor) and energization of the thylakoid membrane.[5] Perturbation of PS2 photochemistry will result in changes in these fluorescence parameters, which can be quantified by the ratio Fx/Fp

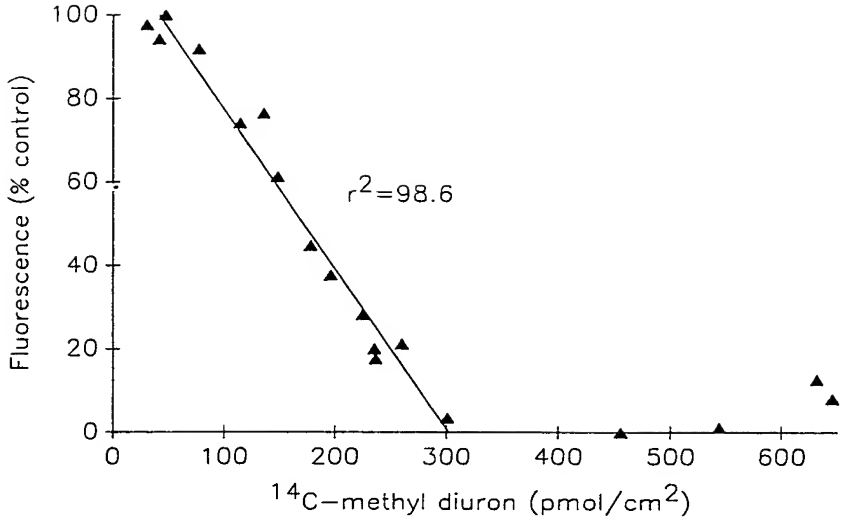

FIGURE 2. Correlation between posttreatment changes in fluorescence (Fx/Fp) and ^{14}C-diuron uptake (expressed on a leaf area basis).

[(Fp − Fi)/Fp] (Figure 1).[4] Any herbicide and PGR effects on PS2 photochemistry and fluorescence are most likely to result from perturbation of photosynthetic electron transport and thylakoid energization and/or impairment of development and repair of photosynthetic membrane components involved in these processes.

The rapid effect of the photosynthetically active herbicide diuron on fluorescence induction in wheat leaves is shown in Figure 1. The response is typical of herbicides which inhibit PS2 photochemistry by binding to the PS2 reaction center D1 polypeptide, preventing electron transfer to PQ and reoxidation of Q_A. Simultaneous fluorescence and ^{14}C-diuron incorporation measurement (Figure 2) indicate that uptake of diuron into the leaves correlates with the fluorescence changes generated. This may result from the fluorescence effect being directly related to diuron binding to the D1 polypeptide, which will be determined by the relative uptake of herbicide. Consequently, fluorescence measurement can be used for the rapid estimation of diuron uptake into leaves and to investigate factors which influence herbicide penetration.

It is well known that the formulation of herbicides with adjuvants can markedly facilitate uptake;[3] however, with few *in vivo* probes of herbicide penetration, the processes involved are neither well understood nor fully optimized. Enhanced herbicide uptake was indicated by a greater fluorescence response from leaves treated with diuron formulated with 0.1% (v/v) Tween 20 (Figure 3). Tween 20 by itself (and other adjuvants used in this study) had no effect on fluorescence emission (data not shown). Removing the leaf epicuticular wax layer resulted in a greater fluorescence response, which was not affected by the inclusion of Tween 20 (Figure 3). These fluorescence results clearly identify the epicuticular wax layer as the major site of resistance to the uptake of diuron and demonstrate that Tween 20 interacts only with this layer when promoting herbicide uptake.

The fluorescence response of leaves treated with diuron was also found to be dependent on the Tween 20 concentration (Figure 4). The maximum effect was observed using approximately 100 mg/l of Tween 20, close to its cmc (Figure 4). Above the cmc, the concentration of the monomeric form of the adjuvant is maximally maintained, suggesting

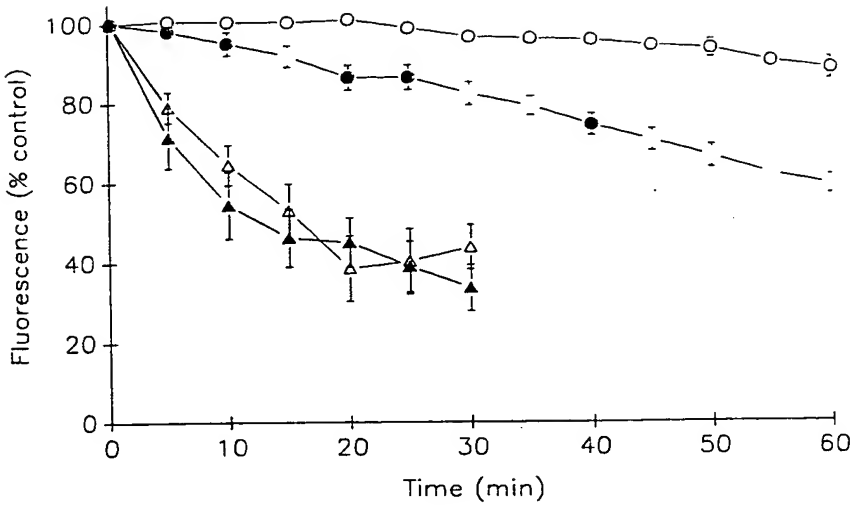

FIGURE 3. Posttreatment changes in the fluorescence induction parameter (Fx/Fp) from wheat leaves treated with diuron (○) and diuron + 0.1% (v/v) Tween 20 (●), and leaves with their wax layer removed and treated with diuron (▲) and diuron + 0.1% (v/v) Tween 20 (△).

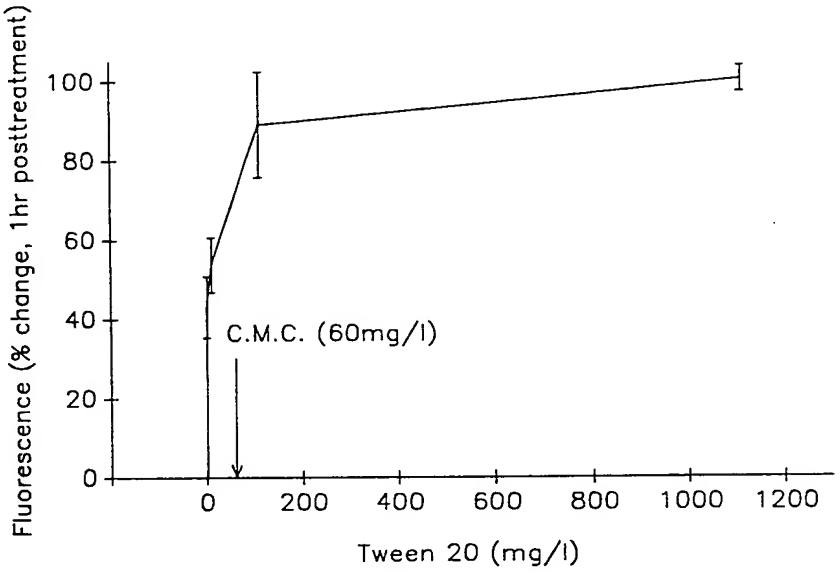

FIGURE 4. Changes in the fluorescence parameter (Fx/Fp) measured from leaves 1 h after treatment with diuron formulated with a range of Tween 20 concentrations.

that the adjuvant is probably most effective at facilitating diuron uptake in its monomeric form. The results also demonstrate that fluorescence measurement can be used for the rapid determination of effective adjuvant concentrations.

Leaves treated with formulations of the PS2 herbicides, phenmedipham (Betanal-E) and bentazon (Basagran), with water and the commercially available adjuvants, Actipron (mineral

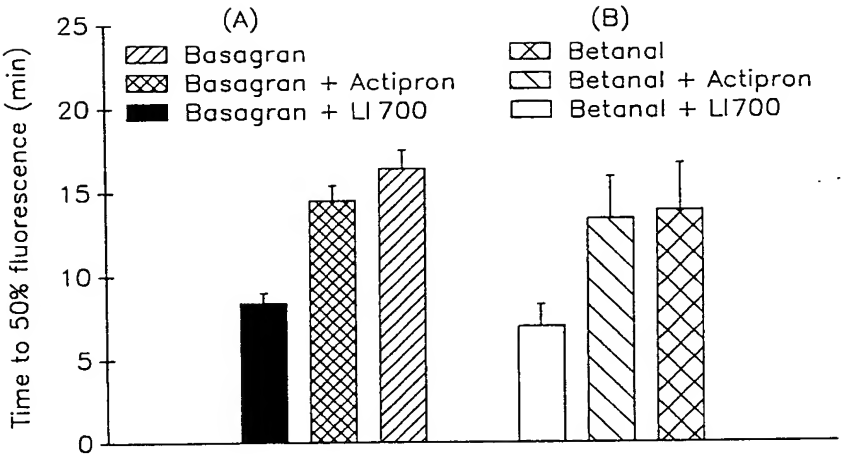

FIGURE 5. Comparison of the effects of (A) water, Actipron (2.5%, v/v), and LI 700 (0.5%, v/v) formulations of Betanal-E [a.i.] (active ingredient) phenmedipham, 1.425 mg/ml) and (B) water, Actipron (1.0%, v/v), and LI-700 (0.5%, v/v) formulations of Basagran (a.i. bentazone, 9.6 mg/ml) on fluorescence emission from treated leaves as estimated from the time to achieve 50% reduction in Fx/Fp.

oil) and LI 700 (acidified soya lecithin), also exhibited different fluorescence responses indicative of different herbicide uptake rates (Figure 5). The most rapid fluorescence responses showed that LI 700 was the most effective with both herbicides at enhancing uptake. The results also demonstrate that these fluorescence kinetics can be employed to monitor the penetration of any herbicide which affects PS2 photochemistry directly.

Changes in the fluorescence emission from immature wheat leaves were also found to relate to the application dosage of the nonphotosynthetic herbicide, glyphosate (unpublished data), and the PGR, chlormequat.[7] These responses were determined 24 h after treatment and were possibly due to some impairment of the development of fully competent photosynthetic membranes in younger tissues.[7] Consequently, it was also possible to screen different formulations of these chemicals with the commercial adjuvants Ethokem, Agral, and LI 700, using fluorescence. Glyphosate effects were most enhanced by Ethokem, whereas LI 700 promoted the most uptake of chlormequat (Figure 6).

In conclusion, despite the need for further investigations to confirm and correctly interpret many of the observations relating to fluorescence changes, these preliminary studies do indicate that chlorophyll fluorescence has potential for use as a rapid, sensitive, noninvasive probe for monitoring factors affecting herbicide and PGR uptake and activity in intact leaves. The technique would also appear to have application for the rapid screening of effective field herbicide and PGR adjuvant formulations.

ACKNOWLEDGMENT

Some of the fluorescence data are reproduced with the kind permission of Newman Agrochemicals Ltd., Barton, Cambs. CB3 7AR, U.K.

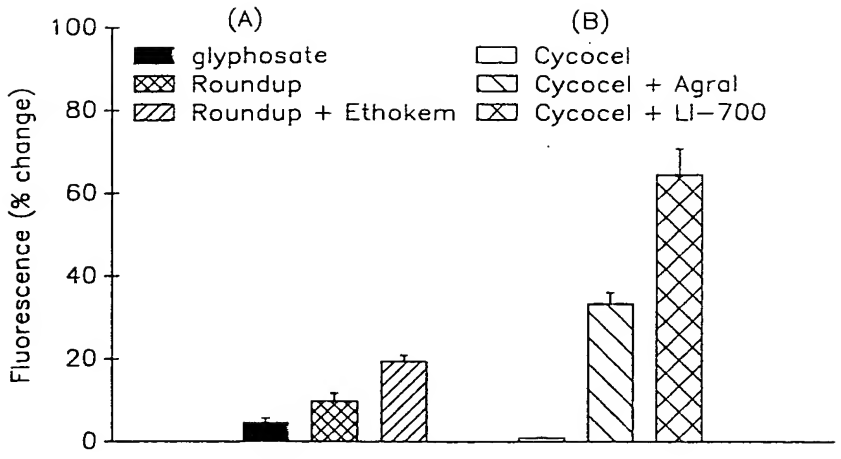

FIGURE 6. Comparison of (A) the effects of glyphosate (3.35 mg/ml), Roundup® (active ingredient [a.i.] glyphosate, 3.35 mg/ml), and Roundup® + 0.5% (v/v) Ethokem, and (B) Cycocel (a.i. chlormequat, 8.04 mg/ml), Cycocel + 0.025% (v/v) Agral, and Cycocel + 0.5% (v/v) LI-700 on fluorescence (Fx/Fp) emission from immature wheat leaves 24 h after treatment.

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Chapter 16

PHOTODEGRADATION AND ABSORPTION OF SETHOXYDIM AS ADJUVANT-INFLUENCED SURFACE EFFECTS

James L. Hazen and Philip J. Krebs

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ABSTRACT

Experimental adjuvants were tank mixed with sethoxydim {2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} and tested on glass slides and whole plants to evaluate their influence on herbicide photodegradation and absorption, respectively.

Adjuvant materials were classified relative to their effect on sethoxydim half-life when exposed to photodegradation conditions in a temperature-controlled ORIGINAL HANAU Suntest Apparatus. Studies on seedling corn (*Zea mays* L.) identified adjuvant materials which greatly enhanced the absorption of sethoxydim from a controlled application of tank mix to the leaf surface.

The standard crop oil concentrate was shown to greatly enhance the rate of sethoxydim degradation on glass.

A superior adjuvant for sethoxydim was formulated from materials thus identified. In addition, certain of these materials were incorporated into new ready-to-dilute (RTD) formulations which contain herbicide and adjuvant.

I. INTRODUCTION

It is well established that adjuvants play an important role in the application of herbicides. Experience shows that successful weed control results when the appropriate adjuvant and herbicide combination are properly applied to the target. An appropriate adjuvant is one which has been selected on its ability to consistently meet the needs of the herbicide formulation with which it is co-applied.

Such needs include physical, chemical, and/or biological modifications. Physical and chemical uses for adjuvant materials are related to sprayability, compatibility, and other water-quality influences. There are many nuances of biological improvement via adjuvants. Some result from consideration of the previous chemical and physical problems, while others result directly from the effect of the application on the target.

In most cases, commercial adjuvants were developed to improve the physical appearance of the spray solution (dense, white emulsion of paraffinic oil) without having a specific pesticide formulation or active ingredient in mind. With few exceptions, oil concentrates are not regulated for activity, compatibility, or consistency of composition. Finding the appropriate adjuvant is unlikely to happen without a serious investigation.

Interest in developing a superior adjuvant system began with the realization that we could not control the quality of crop oil concentrates on the market and that, with the proliferation of such adjuvants, it would be impossible to test and maintain a list of acceptable adjuvants for use with products such as Poast Herbicide®* (sethoxydim).

Examining the adjuvant needs of BASF herbicides required the design of a program to identify adjuvant materials for specific targets and later to evaluate their effect on the herbicide as well as the target. Certain aspects of this research have been previously reported.[1-4]

As the biochemical optimization of our sethoxydim formulation was approached, obvious differences in adjuvant performance between greenhouse and field trials became a concern.

If a population of plants is treated and half of them are placed outside in the direct sunlight while the other group is kept in the greenhouse under similar environmental conditions, except for the lack of UV light, the effect of the UV light exposure can be observed as differences in plant injury.

In the greenhouse, 100 g/ha sethoxydim is active across a variety of species. The activity drops off dramatically on the plants placed outside, especially corn. The investigation to explain these indoor/outdoor performance variations is the basis of this chapter.

* Poast Herbicide is a registered trademark of BASF AG.

II. MATERIALS AND METHODS

A. TEST MATERIALS

Poast Herbicide, Lot SP4040, was used as the source of sethoxydim for glass slide and whole plant studies. Dash Adjuvant*, Lot 86-5, and AGWAY BOOSTER Plus E (no lot number) were used as the standard adjuvants. World Health Organization (WHO) standard 342 ppm synthetic hard water was used as diluent.

Adjuvants were examined as coded materials, identified with BCH . . . S numbers. Many of the tested materials were previously identified in the references as surfactants from series A, B, C, or D, having a subsequent one- or two-digit number (e.g., Surfactant D-7, which later became BCH 815 00S).

B. TEST SOLUTIONS

The tank-mix solutions in the glass slide test were prepared as 0.5 g of Poast Herbicide with 2.5 g of adjuvant brought with acetone to a 100-ml volume in a Class A volumetric flask. This yields a sethoxydim concentration of 1.12 mg/ml, which corresponds to a use rate of 1.17 l/ha of Poast Herbicide with 5.85 l/ha of adjuvant in 187 l/ha of spray solution (1 pint of Poast with 2.5 qt of adjuvant in 20 gal/acre). This adjuvant rate was a bit high; however, the interest was in optimal adjuvant use rates for maximum adjuvant effect. Greater and/or lesser adjuvant rates were examined for certain materials. Solutions were transferred into amber glass bottles for storage.

Tank-mix solutions for the whole plant study were prepared by weighing approximately 0.5 g of Poast Herbicide, Lot SP4040, into a 100-ml Class A volumetric flask, adding 20 ml of water, and mixing prior to adding the adjuvant material, which was weighed (varied amounts, proportional to composition in blend or to field use rate) into the flask and then brought to volume with additional 342 ppm hard water. The content was then transferred to an 118-ml amber bottle to protect the sample from light.

C. ANALYTICAL METHOD

HPLC analysis was accomplished on a REGIS 5-μ ODS-II, 25 cm × 4.6 mm column, with a mobile phase (80:19:1) of acetonitrile, water, and acetic acid, flowing at 1.0 ml/min. The result of a 250-μl injection was detected at a wavelength of 280 nm.

The standard solutions of sethoxydim were prepared by weighing 100.9 mg of 9.9% sethoxydim (active ingredient) weighed into a 250-ml Class A volumetric flask, thus having a concentration of 0.040 mg/ml. A standard curve was generated from dilutions of this stock solution (e.g., 10 ml into 100 was 0.0040 g/ml, and additional stock solution dilutions were 8, 5, 3, and 1 ml into 100 ml, yielding respective concentrations of 0.004 to 0.0004 mg/ml).

D. GLASS SLIDE STUDY

A study of sethoxydim on glass slides was designed to provide information about the effect of adjuvant materials on the rate of photodegradation.

Glass microscope slides were spotted by syringe with 10 μl of test solution applied as a single spot. Treated slides were then exposed for increments of 0, 1, 2, 3, 4, 5, 10, 15, 20, 30, and 40 min. The ORIGINAL HANAU Suntest Apparatus** was operated with cooling to the tray to maintain a surface temperature of 28°C. The fan was on and the energy output level of the lamp was not observed to vary over the test period. Preliminary studies

* Dash Adjuvant is a registered trademark of BASF Corporation.
** DSET Laboratories Inc., Phoenix, AZ 85027.

in the Suntest Apparatus had been conducted to determine the effects of fan, cooling to tray, and exposure time.

Due to limited space on the Suntest tray and the need to compare multiple solutions concurrently, there could be only one slide per tank mix per time interval. Over the course of the entire study, several tank mixes were repeated with very similar results.

At t_0, 10 µl of test solution was transferred onto a glass slide which was then rinsed with 3.0 ml of acetonitrile into a scintillation vial. At each appropriate interval, the slides were individually rinsed with 3.0 ml of acetonitrile, recycled a number of times over the slide surface to ensure adequate flushing of the tank-mix residue into the collection container. The efficiency of glass slide rinsing had been investigated by comparing the sethoxydim rinsing efficiency of ethyl acetate, isooctanol, dimethyl ketone, methanol, and acetonitrile. The latter removed the most sethoxydim and was used for these studies.

Residue collected in this rinsate was directly assayed for sethoxydim. This was possible since the rinse volume and initial sethoxydim applied combined to bring the concentration above the lower detection limit for the analytical method employed. As the mobile phase was 80% acetonitrile, direct injection did not disturb system equilibrium.

Analytical values were normalized against the recovery of sethoxydim from a treated, non-UV-exposed slide. During preliminary studies, attempts were made to incorporate an internal standard to assist with the accurate quantification of sethoxydim; however, thymol did not adequately rinse from the slide due to limited solubility in isooctane, and dipropyl phthalate was noted to decompose when exposed to sunlight. Since the thymol and dipropyl phthalate internal standards had proved to be unreliable, correlation was made to a nonsunlight-exposed, zero-time slide application of the appropriate tank mix.

It was noted that a solvent (Aromatic 150 — EXXON), detectable under the stated chromatographic conditions, was interfering with the t_0 samples since it did not have time to evaporate prior to sample work-up. Nitrogen was used to gently blow dry the t_0 slides. This removed the interfering solvent and was shown not to cause detectable degradation of sethoxydim.

E. WHOLE PLANT STUDY

These studies determined the amount of applied sethoxydim that can be recovered from the surface of a corn plant leaf at increasing time intervals after application of a solution containing a known amount of sethoxydim. (The amount of sethoxydim recovered subtracted from the initial 100% equals the absorbed sethoxydim which is no longer at the leaf surface.)

A preliminary study of sethoxydim on corn leaf surfaces was accomplished to determine the length of exposure time, relative rate of uptake, and leaf rinsing procedures. Some attention was given to confirm that significant sethoxydim decomposition products were not generated in the growth chamber since decomposition had been noted on plants placed outdoors.

Greenhouse-grown corn (Pioneer 3320) plants, ranging from 15 to 23 cm (6 to 9 in) tall at 10 to 12 d after planting (DAP), were used as test specimens. Plants were grown in a 1:1 blend of Norfolk sandy loam/Metro 360* organic medium, amended with lime and fertilizer at agricultural field rates. Light intensity in the greenhouse for initial growth ranged from 240 to 530 $\mu E\ M^{-2}\ s^{-1}$ for supplement light and sunlight (mid-September), respectively. The plant population in 8.2-cm-diameter pots was reduced to one per pot by severing all others at the soil surface before transferring to the growth chamber. The test plants were allowed to acclimate to the growth chamber conditions for 24 h before treatment. Growth chamber conditions were adjusted to less than optimal conditions (20°C and 50% relative humidity rather than the normal 25°C at 85% RH) to encourage expression of adjuvant

* Product of W. R. Grace, Horticultural Products, Cambridge, MA.

TABLE 1
Percent of Initial Sethoxydim Recovered from Glass Slide Surface After 28°C Suntest UV Exposure

Adj.:Seth. ratio	Adjuvant description	Exposure interval (min)									
		1	2	3	4	5	10	15	20	30	40
0:1	None	80	57	47	31	26	14	6	5	—	—
2.50:1	Dash	83	73	60	52	48	24	13	8	—	—
2.50:1	COC	77	63	47	34	30	8	5	0	—	—
1.88:1	BCH 815 00S	76	57	45	35	29	16	8	5	2	0
0.94:1	BCH 828 00S	78	63	50	37	33	16	8	7	0	0
0.94:1	BCH 826 00S	86	72	59	45	36	26	16	10	7	6
0.38:1	BCH 834 00S	85	77	67	60	57	42	30	25	6	4
0.25:1	BCH 836 00S	89	80	69	59	58	41	30	22	13	8
2.50:1	BCH 779 00S	74	53	37	34	18	5	0	0	—	—

effects. Growth chamber light (continuous) was from incandescent and fluorescent sources; the light energy at bench height was measured as 175 to 200 $\mu E\ M^{-2}\ s^{-1}$ with no measurable UV component.

Normal application of a 10-µl test solution onto the horizontal adaxial leaf surface was accomplished as 20 spaced droplets using a Hamilton repeating dispenser (micropipetter) fitted with a Hamilton 10-µl syringe.

At t_0, 10 µl of test solution was spotted onto the leaf surface and immediately rainsed with 3.0 ml of acetonitrile, recycled into a scintillation vial. Two plants were used for each tank-mix time interval. Sethoxydim recovery from intervals of 0.25, 1, 2, 3, 4, 5, and 7 h were normalized to the t_0 recovery value.

III. RESULTS AND DISCUSSION

A. GLASS SLIDE STUDY

Sethoxydim is a very photolabile material, but it is not the only compound with this sensitivity. The half-lives for sethoxydim and many other postemergence graminicides are within the 45- to 180-min range.

We examined the degradation of sethoxydim on glass slides under a nonfiltered UV lamp. In an ORIGINAL HANAU Suntest Apparatus, the half-life of sethoxydim is about 9 min. With the 300-nm cutoff filter in place, the UV energy in this test system is reported to represent the intensity of the sun at noon on the equator. In the real world, the actual half-life is longer, but still short enough to become a major concern.

The first slide study compared the relative rate of sethoxydim decomposition, as Poast Herbicide alone, with Dash Adjuvant, crop oil concentrate (ICI — AtPlus 411F-type), and methylated sunflower oil. In Table 1, most obvious is the fact that crop oil concentrate (COC) and methyl-sunflower oil accelerate the photodecomposition of sethoxydim, while Dash has a minimal effect.

This test also examined the effect of individual components of Dash in the proportions in which they are used in Dash Adjuvant. It can be seen that 834 and 836 improved the stability of sethoxydim, while 815 and 779 slightly increased the rate of decomposition.

In Table 2, the effect on sethoxydim stability from the increase or decrease of an individual Dash component was evaluated. Increasing 834 had no effect, but decreasing 834 slightly reduced the recovery of sethoxydim. There was no improvement in sethoxydim recovery from changing the 836 content. Certain other materials were shown to have a

TABLE 2
Percent of Initial Sethoxydim Recovered from Glass Slide Surface After 28°C Suntest UV Exposure

Adj:Seth. ratio	Adjuvant description	Exposure interval (min)									
		1	2	3	4	5	10	15	20	30	40
0:1	None	88	81	71	62	59	46	32	20	16	9
5:1	Dash	86	80	67	61	58	40	29	21	11	6
5:1	COC	75	62	50	35	29	10	5	0	0	0
0.38:1	BCH 834 00S	89	78	71	62	55	42	34	24	15	9
0.25:1	BCH 834 00S	87	79	66	58	56	37	21	12	11	8
1.00:1	BCH 834 00S	87	80	71	61	58	45	31	23	15	9
0.25:1	BCH 836 00S	87	76	65	54	52	34	25	16	12	7
0.13:1	BCH 836 00S	87	77	66	55	57	31	22	13	8	5
1.00:1	BCH 836 00S	87	—	66	52	50	41	23	16	9	5
0.25:1	BCH 836 05S	81	78	64	57	52	38	27	19	12	7
0.25:1	BCH 770 00S	88	78	63	57	55	37	20	11	8	6
0.94:1	BCH 828 00S	79	69	51	42	32	19	9	4	0	0
0.50:1	BCH 890 00S	87	80	67	58	51	40	27	16	9	5
0.50:1	BCH 842 00S	86	77	67	58	55	39	29	20	12	9
0.50:1	BCH 843 00S	89	81	72	62	60	45	31	19	12	7

TABLE 3
Percent of Initial Sethoxydim Recovered from Glass Slide Surface After 28°C Suntest UV Exposure

Adj.:Seth. ratio	Adjuvant description	Exposure interval (min)									
		1	2	3	4	5	10	15	20	30	40
0:1	None	88	81	71	62	59	46	32	20	16	9
5:1	COC	75	62	50	35	29	10	5	0	0	0
5:1	Dash	86	80	67	61	58	40	29	21	11	6
5:1	Dash	87	79	68	59	55	32	22	13	8	4
5:1	Methyl Sunflowerate	79	67	52	42	35	21	9	5	0	0
0.5:1	Methanol	88	80	68	63	54	39	21	12	10	3
0.5:1	*n*-Octanol	93	81	73	66	57	46	35	26	17	7
0.5:1	*t*-Butanol	87	79	64	59	55	41	26	17	6	5
0.5:1	*p*-Aminobenzoic acid	93	90	79	69	74	55	45	37	29	18
0.5:1	Propionic acid	85	81	67	56	52	36	26	16	7	5
0.5:1	Benzoic acid	89	83	72	67	62	47	46	20	13	11
0.5:1	α-Tocopherol acetate	84	74	57	45	40	38	20	12	8	4
0.5:1	Ascorbyl palmitate	91	86	76	70	68	57	41	38	21	19
0.5:1	Ascorbic acid	91	84	76	70	66	51	42	29	20	15
0.25:1	Uvinul D-49	95	94	88	81	78	61	57	48	29	25
1.00:1	Uvinul D-50	97	94	94	90	87	84	76	69	58	51
0.50:1	Uvinul D-50	97	94	92	88	85	79	74	69	60	55
0.05:1	Uvinul D-50	93	92	85	79	76	66	66	35	33	32
0.01:1	Uvinul D-50	92	83	81	72	71	53	45	33	21	13

similar effect on the degradation of sethoxydim and could possibly function as alternate components.

In the next series, several UV absorbers, alcohols, and antioxidants were checked at rates equal to 10% of the nominal adjuvant composition. Table 3 presents data to indicate that UVINUL®* D-50, PABA, and ascorbyl palmitate improved the UV stability of se-

* UVINUL is a registered trademark of BASF AG.

TABLE 4
Percent of Initial Sethoxydim Recovered from Glass Slide Surface After 28°C Suntest UV Exposure. Effect of 300-NM Cutoff Filter

Adj.:Seth. ratio	Adjuvant description	Exposure interval (min): filter/no filter[a]					
		1	3	5	10	20	30
0:1	None	97/89	92/71	89/58	81/43	71/18	64/12
5:1	Dash	94/87	89/68	84/55	73/32	57/13	43/8
1.88:1	BCH 815 00S	97/81	90/57	85/41	74/28	58/6	43/5
0.38:1	BCH 834 00S	96/91	92/76	89/70	81/57	74/38	63/27
0.25:1	BCH 836 00S	96/92	94/83	90/75	80/56	71/41	62/30
2.50:1	BCH 779 00S	94/86	91/69	87/59	80/45	73/21	63/16

[a] Data generated with/without 300-nm UV cutoff filter in Suntest apparatus.

thoxydim; α-tocopherol acetate did not. These responses indicate that the decomposition mechanism is not related to steric hindrance or amphipaths. Antioxidants and UV inhibitors significantly retard the photodegradation of sethoxydim.

Table 4 contrasts the effect of various adjuvant components on sethoxydim stability when the Suntest Apparatus is operated with a 300-nm cutoff filter in place, compared to the norm for this study which did not employ this filter. The energy below 300 nm obviously accelerates sethoxydim decomposition. This indicates that the actual rate of decomposition is substantially less than the nonfiltered data would suggest. Sethoxydim surface degradation by UV light is actually enhanced by COC.

B. WHOLE PLANT STUDY

Two major events occur once the spray droplets have been applied to the target surface: photodegradation and uptake. We have uptake removing herbicide from the leaf surface and photodegradation decomposing the herbicide which remains on the surface. The longer the herbicide remains at the surface, proportionately less potential activity remains for uptake. This reduces the chance for a weed to absorb the lethal dose needed for control.

Two options are available: protect the active ingredient on the surface with a suitable UV protectant and/or increase its rate of uptake by the plant. It is important to get the herbicide into the plant as quickly as possible. The cuticle is known to protect the plant against the effects of UV light; it will do the same for a herbicide that has penetrated the cuticle.

Whole plant studies examined the effect of adjuvants on the absorption of sethoxydim by corn plant leaf. The sethoxydim applied minus the sethoxydim recovered from the leaf surface suggests the amount of sethoxydim absorbed (and/or decomposed).

As shown in Table 5, recovery of sethoxydim from the surface levels out after 3 h, indicating a very slow rate of uptake from the Poast Herbicide formulation applied without any adjuvant. After 7 h, 40% of the initial sethoxydim was left on the surface and significant degradation products were noted. COC improved the rate of sethoxydim uptake, but was no match for Dash Adjuvant. Methylated sunflower oil increased the uptake about as much as BCH 815 00S. BCH 834 00S had significant uptake enhancement, but less than BCH 815 00S.

The last study in this series presents, in Table 6, the sethoxydim absorption for Poast Herbicide when tank mixed with oil concentrate (0.9 l) or with Dash (0.9 l), compared to the adjuvant-containing Poast Plus® Herbicide* formulation. Poast alone and a 120 g/l

* POAST PLUS Herbicide is a trademark of BASF Corporation.

TABLE 5
Percent of Initial Sethoxydim Recovered from Surface of Corn Leaf After 20°C Growth Chamber Exposure

Adj.:Seth. ratio	Adjuvant description	Exposure interval (h)						
		0.25	1	2	3	4	5	7
0:1	None	82	74	61	50	53	53	44
2.50:1	Dash	67	24	8	0	0	0	0
2.50:1	COC	81	62	36	24	8	10	4
0:1	None	87	73	65	60	54	55	39
1.25:1	BCH 815 00S	80	22	8	7	6	0	0
1.25:1	BCH 834 00S	87	77	53	49	37	20	12
0:1	None	90	79	64	62	51	44	42
1.25:1	BCH 836 00S	83	63	23	9	5	0	0
1.25:1	BCH 779 00S	91	82	67	58	52	38	24
0:1	None	91	86	77	73	64	59	45
12.5:1	BCH 778 00S	54	42	39	43	40	26	27
2.50:1	Methyl Sunflowerate	79	46	13	8	6	0	0

TABLE 6
Percent of Initial Sethoxydim Recovered from Surface of Corn Leaf After 20°C Growth Chamber Exposure

Tank-mix description	Exposure interval (h)						
	0.25	1	2	3	4	5	7
0.47 l Poast Herbicide alone	82	74	61	50	53	53	44
0.47 l Poast + COC	81	62	36	24	8	10	4
0.47 l Poast + Dash Adjuvant	67	24	8	0	0	0	0
0.71 l Poast Plus Herbicide	62	18	6	2	0	0	0
0.71 l Control[a]	64	53	51	49	31	27	11

[a] Control is 120 g/l sethoxydim with 20 g/l emulsifier in Aromatic 150 solvent. Poast Plus is 120 g/l and Poast is 180 g/l sethoxydim, respectively.

sethoxydim control are included for comparison. The Poast Plus formulation afforded the same enhanced rate of absorption as with a tank mix of Poast and Dash.

After 2 h, oil concentrate still has 36% of the applied sethoxydim at the surface (64% absorption), while Poast Plus has effected absorption of 94% of the herbicide.

IV. CONCLUSIONS

- Some adjuvant materials affect the photolability of sensitive herbicides.
- An optimal adjuvant composition can enhance herbicide uptake without affecting the rate of photodecomposition.
- Every adjuvant has an optimal use rate.

Light quality plays a significant role in limiting the efficacy of sethoxydim, and this effect is closely related to the rate of sethoxydim uptake. If a set of grass species (corn, green foxtail [*Setaria viridis* (L.) Beauv.], crabgrass [*Digitaria* spp.], and broadleaf signalgrass [*Brachiaria platyphylla* (Griseb.) Nash]) are collectively rated for the amount of herbicide required to yield some level of control across all species, comparison of treatments becomes somewhat easier. Using an inside/outside posttreatment test, the effect of sunlight

can be expressed in terms of herbicide required for 95% weed control (GR_{95}). In the case of sethoxydim without adjuvant, there is nearly a threefold loss in efficacy when exposed to UV light relative to nonexposed plants.

Looking at whole plant efficacy, the outside activity of sethoxydim in the presence of Dash is maintained at levels seen in the absence of UV light. This does not happen with COC. The outside plants exposed to UV require more sethoxydim for the same level of control when treated with oil concentrate rather than Dash Adjuvant.

COC actually increased the photodegradation of sethoxydim in the presence of UV light while having an inadequate effect on uptake. Dash Adjuvant significantly increased the rate of uptake while having a minimal effect on UV photodegradation. Thus, with Dash increasing the rate of foliar uptake while not increasing the rate of photodegradation, there is a dramatic effect on the efficacy of sethoxydim compared to COC.

This approach to investigating the adjuvant requirements for sethoxydim has resulted in the development of a superior adjuvant which meets the needs of the active ingredient and formulation. We believe that all products deserve this consideration of biochemical optimization.

ACKNOWLEDGMENTS

The authors appreciatively recognize the dedicated and skillful work of Tanya E. FitzGerald and Thomas Byrne. Dr. Paul S. Zorner provided helpful suggestions regarding the importance of UV degradation as a potential factor in adjuvant-modified uptake. Thanks also to his staff members for sharing supporting data and for ensuring the availability of plants for our study.

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Chapter 17

THE INFLUENCE OF ULTRAVIOLET LIGHT ON THE PHYTOTOXICITY OF SETHOXYDIM TANK MIXTURES WITH VARIOUS ADJUVANTS

D. McInnes, K. Neil Harker, Robert E. Blackshaw, and William H. Vanden Born

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ABSTRACT

Experiments were conducted at three locations in Alberta to evaluate the effect of photodegradation of sethoxydim {2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one}. The degrading effect of ultraviolet radiation (UV, 300 to 400 nm) was investigated under field conditions as measured by the phytotoxic activity of sethoxydim on preselected samples of volunteer barley (*Hordeum vulgare* L.). Physical screening treatments were employed to control the spectral quality of radiant energy impinging on sethoxydim-treated plants. Several spray adjuvants (oil concentrate, ammonium sulfate, BAS 815, BAS 890) were also examined in mixtures with sethoxydim to determine their influence on sethoxydim activity on barley. Visible light (400 to 700 nm) had little or no influence on sethoxydim activity. However, exposure to UV radiation often dramatically reduced the activity of sethoxydim. The inclusion of adjuvants in the spray mixture counteracted some of the deleterious effects of UV radiation. Of the adjuvants tested, BAS 815 or BAS 890 most effectively preserved sethoxydim activity in the presence of UV radiation.

I. INTRODUCTION

The herbicide sethoxydim controls a broad range of grass weeds in numerous broadleaved crops.[10] The ability of sethoxydim to control both annual and perennial grasses under a wide variety of environmental conditions with excellent crop tolerance has prompted producers of broadleaved crops to make the herbicide a major part of their weed control program. In Canada (1988), approximately 20% of the canola (*Brassica napus* L., *B. campestris* L.) acreage and 80% of the flax (*Linum usitatissimum* L.) acreage that is treated with herbicides is treated with sethoxydim.

Those involved in research with sethoxydim may be aware of some of the interesting characteristics of the herbicide. Adjuvants, particularly oil concentrates and more recently ammonium sulfate,[2,7] significantly improve sethoxydim activity. There are also reports which indicate that sethoxydim is less stable in the presence of heat and light, and that it is rapidly metabolized by plants. Campbell and Penner[1] observed that after 24 h, less than 2% of applied sethoxydim existed in susceptible and tolerant plants. They noted that seven of nine metabolites in the plants cochromatographed with thermal- and phototransformation products of sethoxydim. Several authors have demonstrated photodecomposition of other herbicides in aqueous solutions.[3,4,6,12] In addition, sethoxydim often exhibits superior herbicide activity under greenhouse or other controlled conditions when compared to field conditions. These observations led to the hypothesis that sethoxydim activity in the field may be significantly influenced by solar radiation. Tanaka et al.[11] have noted that only a limited amount of research has been conducted in the area of photodegradation of herbicides, and even less on the specific effects of herbicide additives on photodegradation. This project was undertaken with the following objectives: (1) to determine the effectiveness of several adjuvants with sethoxydim, (2) to determine if UV radiation alone influences sethoxydim activity, and (3) to determine the interaction of UV screening treatments on sethoxydim in the presence and absence of several adjuvants.

II. MATERIALS AND METHODS

Field experiments were conducted at three locations (Lacombe, 52.30° N 113.42° W; Olds, 51.50° N 114.06° W; and Lethbridge, 49.43° N 112.48 ° W) in Alberta, Canada in 1988. Barley was cross-seeded in flax to simulate an infestation of volunteer barley. The experiments were designed as split plots with seven herbicide treatments (Table 1) as main

TABLE 1
Notations of the Herbicide/Adjuvant Treatments to be Used in the Tables and Figures

Notation	Sethoxydim	Adjuvants
Untr	—	—
Stan	0.25	1% OC[a] + 2.0 AS[b]
−Adj	0.15	—
Oil	0.15	1% OC
O + AS	0.15	1% OC + 2.0 AS
815	0.15	1% BAS 815
890	0.15	1% BAS 890

[a] OC, paraffin-base mineral oil (83%) and surfactant (17%).
[b] AS, BASF liquid ammonium sulfate (490 g/l).

TABLE 2
Notations of the Screening Treatments to be Used in the Tables and Figures

Notation	Screen	Transmission
Open	—	Complete
OP1 (+UV)	Clear acrylite[a]	Both UV and visible
OP2 (−UV)	Clear acrylite	Only visible
Mirror	Reflective acrylite	Neither

[a] Chemacryl Plastics Ltd., Rexdale, Ontario, Canada.

plots and four UV screening treatments (Table 2) as subplots replicated four times. At the time of treatment, 15 uniform barley plants were ringed in each subplot for subsequent barley harvest. A CO_2-pressurized sprayer that delivered a water volume of 100 l/ha at 276 pKa was used to apply the herbicide/adjuvant treatments when the barley was in the three- to five-leaf stage with zero to two tillers. All spray treatments were applied at approximately 11:00 a.m. Mountain Standard Time. The UV screening treatments were applied immediately after the spray treatments and were left covering the plots until sunset (approximately 11 h elapsed time). An LI-1800 LI-COR portable spectroradiometer was used to measure the electromagnetic spectrum (from 300 to 850 nm) transmitted through the screening treatments. Three measurements for each 10-nm portion of the spectrum (300 to 850 nm) were recorded, and the average is presented in Figure 1 (transmission values are expressed as the percent of the open [no screen] treatment). The following ratings and parameters were also measured: visual barley control ratings, barley fresh weight, barley head counts, barley plant counts, barley mortality, and flax fresh weight. All the data were subjected to an analysis of variance and means were separated either by Duncan's multiple range test ($p < 0.05$) or by single degree-of-freedom contrasts with their associated probabilities.

III. RESULTS AND DISCUSSION

A. EFFECTIVENESS OF THE PHYSICAL SCREENS

Spectroradiometer measurements confirmed that the UV screening treatments were working according to specifications. The OP1 and OP2 screens transmitted at least 90% of the

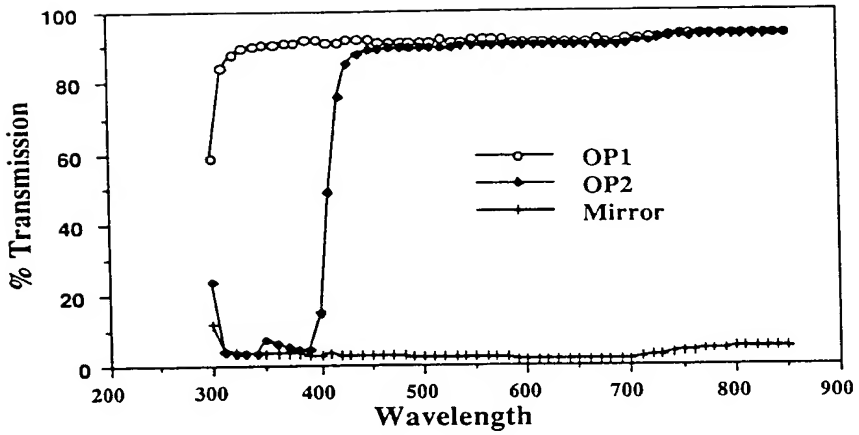

FIGURE 1. Transmission of radiant energy as determined by spectroradiometer readings from 300 to 850 nm; PAR (400 to 700 nm) with no screen = 1965 $\mu E\ m^{-2}\ s^{-1}$. Values are expressed as a percent of the open (no screen) treatment to yield percent transmission.

visible light (400 to 700 nm), and the mirror treatment screened out all direct visible light (Figure 1). The OP2 screen also screened out virtually any UV radiation detected under the open and OP1 screening treatments (300 to 400 nm). Temperature and relative humidity under the different screens did not vary among screening treatments.

B. LOCATION ANALYSIS

Only barley fresh weight data will be presented to indicate the various treatment effects; the analysis of other parameters led to similar conclucions. Location by treatment interactions were significant ($p < 0.01$); therefore, locations were analyzed separately and are presented separately. Most data will be presented without averaging over herbicide or screening treatments, since all herbicide treatment by screen interactions were significant ($p < 0.01$).

C. OVERALL HERBICIDE ACTIVITY

In general, barley control was good at all locations with most herbicide treatments (Table 3). Most adjuvant treatments with the low rate of sethoxydim (0.15 kg/ha) gave similar barley control to the standard treatment of sethoxydim (0.25 kg/ha with oil concentrate 1% [v/v] and ammonium sulfate [2.0 kg/ha]). Without any adjuvant, sethoxydim at 0.15 kg/ha did not provide adequate barley control.

D. VISIBLE LIGHT EFFECTS

Our results clearly indicate that screening out visible light in addition to UV light does not further enhance sethoxydim activity. At Lethbridge, none of the sethoxydim/adjuvant treatments were significantly affected when visible light was screened out in addition to UV radiation (Figure 2). The minus adjuvant treatment had the lowest p-value for the contrast between the OP2 (−UV) and the Mirror (−UV, −visible) at $p = 0.38$; p-values for the remaining sethoxydim/adjuvant treatments were all ≥ 0.84. Data from the other two locations confirmed that visible light did not significantly influence sethoxydim activity (data not shown). A major part of the remaining data (Figures 3 to 5) indicate that UV radiation alone can dramatically reduce the phytotoxicity of sethoxydim applications under field conditions. Therefore, UV radiation was much more detrimental to sethoxydim activity than visible light. These results agree with those of Harrison and Wax.[8]

TABLE 3
Summary of Barley Control with Sethoxydim at Three Locations. Means are Averaged Over the Four Screening Treatments

Treatment	Percent control[a]		
	Lacombe	Olds	Lethbridge
Untr	0 a	0 a	0 a
Stan	85 c	100 c	99 d
−Adj	38 b	68 b	30 b
Oil	74 c	96 c	66 c
O + AS	75 c	100 c	91 d
815	79 c	99 c	100 d
890	78 c	100 c	97 d

Note: Means within a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

[a] Percent control = 100 − [trt. fresh wt/untrt. fresh wt × 100].

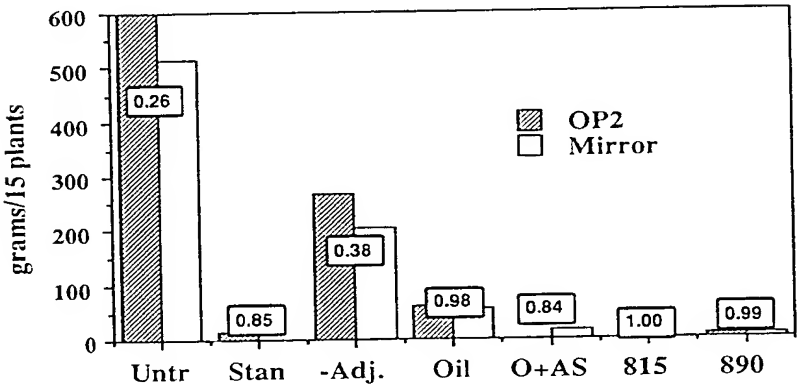

FIGURE 2. The effect of OP2 (−UV) and Mirror (−UV, −visible) screening treatments within individual herbicide/adjuvant treatments on barley fresh weight (Lethbridge). Numbers within or above individual herbicide/adjuvant treatments on the bargraph are *p*-values for the OP2 vs. Mirror contrast.

E. REMOVAL OF UV RADIATION VS. THE ADDITION OF ADJUVANTS

Comparing sethoxydim applied without adjuvants or UV radiation to the same treatment with UV radiation leads to the obvious conclusion that UV radiation has a detrimental effect on sethoxydim activity (Figures 3 to 5). The question then arises: is it possible to retain a high level of sethoxydim activity when adjuvants are applied with sethoxydim in the presence of UV radiation?

Generally, sethoxydim activity was somewhat greater with adjuvants in the presence of UV radiation than if UV radiation was removed and sethoxydim was applied without adjuvants. (That is not to say, as we will discuss below, that adjuvants have value only in overcoming the effects of UV radiation.) At Lacombe and Olds (Figures 3 and 4), the above

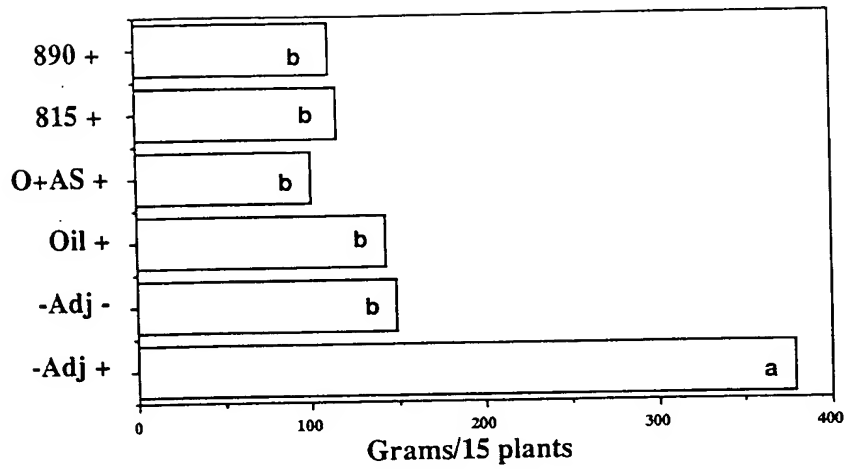

FIGURE 3. The effect of sethoxydim with adjuvants and with UV radiation (OP1; designated as "+" after adjuvant notation) vs. sethoxydim without adjuvants and without UV radiation (OP2; designated as "−" after adjuvant notation) on barley fresh weight (Lacombe). Bargraph portions with the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

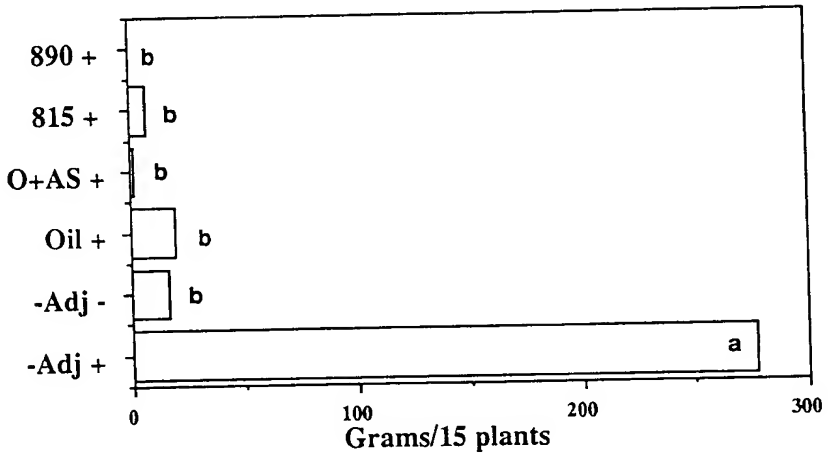

FIGURE 4. The effect of sethoxydim with adjuvants and with UV radiation (OP1; designated as "+" after adjuvant notation) vs. sethoxydim without adjuvants and without UV radiation (OP2; designated as "−" after adjuvant notation) on barley fresh weight (Olds). Bargraph portions with the same letter are not significantly different according to Duncan's multiple range rest at the 5% level.

comparison was not statistically significant, and all adjuvants provided a similar level of sethoxydim activity. However, at Lethbridge (Figure 5), adding BAS 815 or BAS 890 to sethoxydim provided better control than did screening out UV radiation. This suggests that specific adjuvants may add some additional activity to sethoxydim which cannot be accounted for by simply screening out UV radiation.

This study was not designed to determine the individual mechanisms of specific adjuvants. The adjuvants used herein probably increased both the amount and rate of sethoxydim

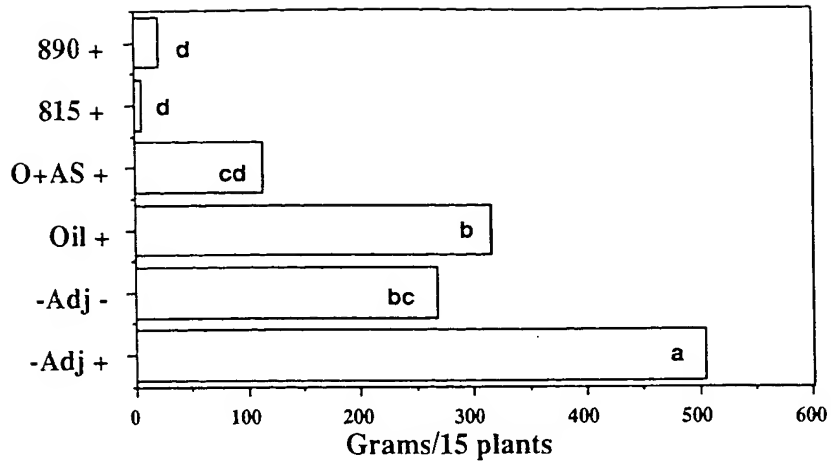

FIGURE 5. The effect of sethoxydim with adjuvants and with UV radiation (OP1; designated as "+" after adjuvant notation) vs. sethoxydim without adjuvants and without UV radiation (OP2; designated as "−" after adjuvant notation) on barley fresh weight (Lethbridge). Bargraph portions with the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

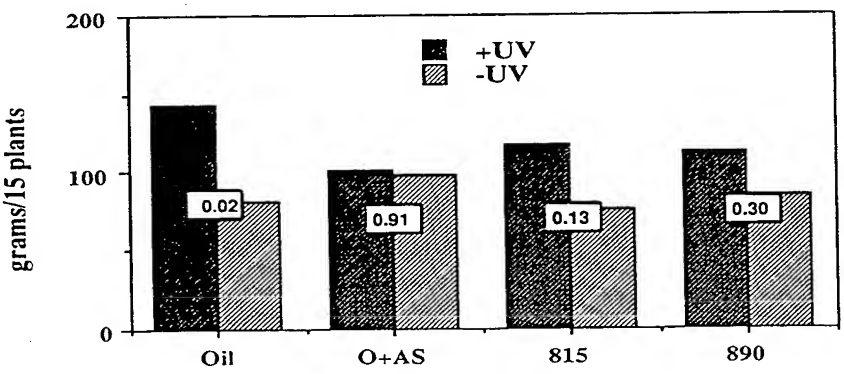

FIGURE 6. The influence of ultraviolet radation (+UV = OP1, −UV = OP2) on sethoxydim/adjuvant treatments on barley (Lacombe). Numbers within or above individual herbicide/adjuvant treatments on the bargraph are *p*-values for the +UV (OP1) vs. −UV (OP2) contrast.

absorption.[5,9,13] It is obvious that the former mechanism would increase the activity of a sethoxydim treatment. However, in the case of sethoxydim, the latter mechanism could also markedly increase sethoxydim activity by decreasing the time the spray mixture would be directly exposed to UV radiation on leaf surfaces (i.e., UV protection by avoidance).

The least effective adjuvant in the current study was oil concentrate. Specific contrasts within individual adjuvant treatments indicated that the activity of the oil concentrate/sethoxydim treatment could be increased when UV radiation was screened out. In Lacombe (Figure 6) and Lethbridge (Figure 8), the *p*-values of the above contrasts were 0.02 and 0.001, respectively. At Olds (Figure 7), all of the treatments controlled the barley so well that there was little room for enhancement by screening of UV radiation ($p \geq 0.65$). These results suggest that the absorption benefits of oil concentrates may be partially negated by

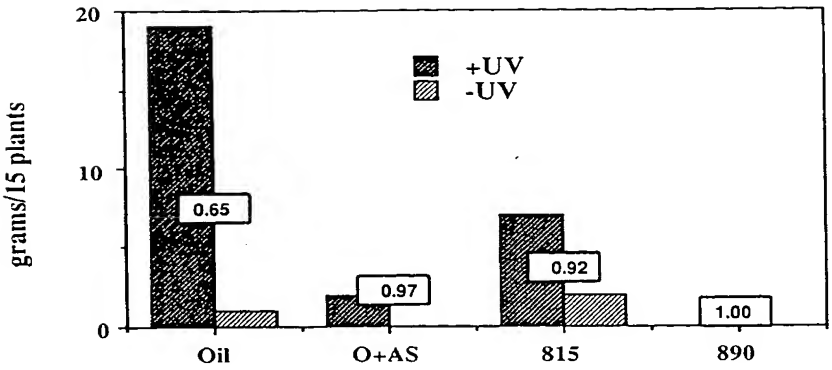

FIGURE 7. The influence of ultraviolet radiation (+UV = OP1, −UV = OP2) on sethoxydim/adjuvant treatments on barley (Olds). Numbers within or above individual herbicide/adjuvant treatments on the bargraph are *p*-values for the +UV (OP1) vs. −UV (OP2) contrast.

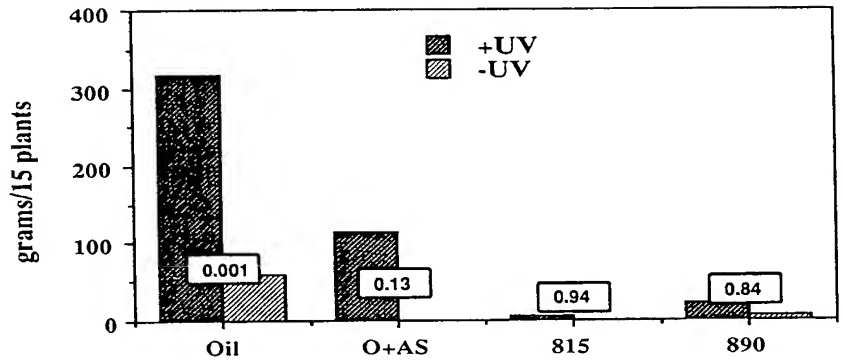

FIGURE 8. The influence of ultraviolet radiation (+UV = OP1, −UV = OP2) on sethoxydim/adjuvant treatments on barley (Lethbridge). Numbers within or above individual herbicide/adjuvant treatments on the bargraph are *p*-values for the +UV (OP1) vs. −UV (OP2) contrast.

the tendency of oil concentrates to increase herbicide photodegradation. Tanaka et al.[12] reported that monuron [*N'*-(4-chlorophenyl)-*N,N*-dimethylurea] photolysis increased in the presence of nonionic surfactants. Harrison and Wax have demonstrated that the photolysis rates of 2,4-D[(2,4-dichlorophenoxy)acetic acid], bentazon [3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide], and haloxyfop {2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]-phenoxy]propanoic acid} were increased in the presence of oil concentrates.[8] No significant increases in sethoxydim activity were apparent when UV radiation was screened out of the other adjuvant treatments (Figures 6 to 8). These adjuvant treatments (O + AS, 815, 890) probably effect such rapid sethoxydim penetration that UV radiation effects on sethoxydim are minimal.[5,9,13]

IV. CONCLUSIONS

Several conclusions can be drawn from this study.

1. Adjuvants enhance low rates of sethoxydim (0.15 kg/ha) on barley.
2. Screening out UV radiation improves the activity of sethoxydim without adjuvants.

3. With UV radiation screened out, there appears to be no further enhancement of sethoxydim activity when visible light is also screened out (UV degradation vs. photo or visible light degradation).
4. With UV radiation screened out, the activity of sethoxydim alone often parallels that of sethoxydim in the presence of UV radiation with adjuvants (depending on the adjuvant). In the presence of some adjuvants, particularly oil concentrate alone, sethoxydim activity can be increased by screening out UV radiation.
5. With respect to sethoxydim activity, the adjuvants used in this study rank as follows:

$$\text{Oil} < \text{O} + \text{AS} \leq 815 = 890.$$

Further studies are necessary to determine whether the adjuvants in this study facilitated an increased speed and amount of sethoxydim penetration and/or if the adjuvants altered the access or activity of the UV light to the herbicide solution.

ACKNOWLEDGMENT

We thank Dr. J. Mason Robertson for his critical review of the manuscript.

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Chapter 18

STABILITY AND ACTIVITY OF CLETHODIM AS INFLUENCED BY pH, UV LIGHT, AND ADJUVANT

David C. Bridges, Linford N. Falb, and Albert E. Smith

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ABSTRACT

Research indicated that abiotic transformation, or degradation, of clethodim {(*E*,*E*)-(±)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} contributes to reduced performance under some environmental conditions. Clethodim was labile in acid aqueous conditions and in UV light. Clethodim loss in UV light was 100, 100, and 99% at pH 5, 6, and 7, respectively. Photodegradation of clethodim was enhanced by the addition of any of five adjuvants. Differences in degradation rate were observed among the adjuvants. Polar degradation products of clethodim were found to be active on sorghum (*Sorghum bicolor* L.) if adjuvant was used. Foliar absorption of ^{14}C-clethodim and polar degradation products of ^{14}C-clethodim differed with the five adjuvants. Results indicate that adjuvant selection is a major factor in determining the stability and activity of cyclohexanedione herbicides such as clethodim.

I. INTRODUCTION

Adjuvants, any material added to a herbicide solution that alters the physical or chemical properties of the solution and which results in modified activity, have been used as an integral part of weed management for many years.[15] Adjuvants have been divided into several classes based on their use and purpose. Among these adjuvant classes are surfactants, emulsifiers, deflocculants, wetting agents, crop oils, and phytobland petroleum or crop oil concentrates. Adjuvants have also been classified as solution modifiers, utility adjuvants, and activators.[8] Activators include surfactants, crop oils, and crop oil concentrates.

The use of crop oils began in the late 1950s and early 1960s to enhance the postemergence activity of atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine].[13,16] More recently, use of crop oil concentrates has increased, especially with the advent of selective postemergence-applied grass herbicides during the early-to-mid 1980s.

Selective postemergence-applied grass herbicides are commonly applied with the addition of various crop oil concentrates at rates of 1 to 2 l/ha. Research has clearly demonstrated differential herbicide efficacy when phenoxy-alkyl or cyclohexanedione-derived herbicides are used with nonionic surfactants, petroleum oil concentrates plus nonionic surfactants, and vegetable oils.[1,9-11,14] These adjuvants are used to improve spray delivery and to increase foliar absorption of herbicides.

Currently, several proprietary products are being marketed. Some are claimed to enhance the performance of grass herbicides. The authors and other researchers[17,18,20,21] have found as little as 17% and generally no more than 40% absorption of sethoxydim {2-[1-ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} applied foliarly in the absence of crop oil concentrate. Therefore, it is not surprising that differential efficacy occurs among adjuvants. Reports indicate that D7, an active ingredient in Dash®,* a commercial, proprietary adjuvant product enhances the foliar uptake of sethoxydim and may render Dash® a superior adjuvant compared to crop oil concentrate for use with sethoxydim.[18,24]

Results of field tests indicate that under some environmental conditions, johnsongrass (*Sorghum halepense* L., Pers.) control with sethoxydim and clethodim is greater with Dash than with crop oil concentrate, especially when these herbicides are tank-mixed with bentazon [3-(1-methylethyl)-(1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide].

Mechanisms responsible for herbicide antagonism have been classed as biochemical, competitive, physiological, and chemical.[12] Chemical antagonisms occur when the antagonist

* Registered trademark of BASF Corp., 100 Cherry Hill Rd., Parsippany, NJ 07054.

reacts with the herbicide in a way that renders the herbicide less active. Sethoxydim efficacy is reduced in the presence of bentazon presumably by the exchange in Na^+ ions with the H^+ from the hydroxyl group of sethoxydim, which yields the Na salt of sethoxydim.[18,19,22,23] Furthermore, Li, K, Cs, Ca, and Mg salts have also been reported to decrease ^{14}C-sethoxydim absorption,[18] and the addition of calcium hydroxide has been shown to have a mild antagonistic effect on the efficacy of clethodim.[1]

Salt effects have been demonstrated to play an important role in the absorption and efficacy of cyclohexanedione herbicides. Results of some research indicate that solution pH may significantly affect the efficacy of these herbicides. Sethoxydim and clethodim are weak organic acids having pK_a values of approximately 4.6. Research has shown that the uptake and biological activity of weak organic acids increases as the pH of the applied solution approaches the pK_a of the acid involved. Application of these acid herbicides in relatively acidic solutions restricts the ionization of the acid, which is more lipophilic and penetrates the leaf cuticle more readily than does the corresponding salt(s) of the acid. Chow and MacGregor[4] reported that sethoxydim solutions at pH 4 and 6 were slightly more efficacious than solutions at pH 8, but when ammonium sulfate was added, the pH effect was not observed. Bridges[1] reported little or no pH dependence with sethoxydim or clethodim over a pH range of 3.5 to 7.5. Evans et al.[5] reported that enhanced sethoxydim uptake with Dash® may be partly attributed to the acidic pH of the adjuvant, which retains the herbicide in the protonated form, thus permitting ion trapping.

As previously described, these processes involve the reaction of the herbicide with an antagonist which renders the herbicide less efficacious. Another potential mechanism that would account for reduced herbicide efficacy is abiotic degradation of the herbicidally active component of a spray solution. Sethoxydim has been shown to undergo physical degradation or abiotic transformation,[3,21] with relatively small amounts of the herbicide being absorbed. Therefore, the purpose of our research program over the past 2 years has been to determine if factors other than those previously described control the efficacy of cyclohexanedione herbicides such as clethodim. The objectives were to (1) determine the stability of clethodim under various environmental conditions, (2) identify potential mechanisms of physical degradation, and (3) determine the effect of various adjuvants on the stability of clethodim.

II. MATERIALS AND METHODS

Four series of experiments were conducted. The first series was conducted to quantify the acid- and/or photocatalyzed degradation of clethodim. The second series was conducted to determine the influence of various adjuvants on the rate of photodegradation of clethodim. The third series of experiments was conducted to determine the relative activity of clethodim and its degradation products. The fourth series of experiments was conducted to measure the influence of various adjuvants on the absorption of clethodim and degradation product(s) into sorghum seedlings.

A. MECHANISM AND RATE OF DEGRADATION

Experiments were performed with clethodim at 50 ppm in buffered aqueous solutions at pH 5, 6, and 7. They were conducted with both technical-grade (35% active ingredient, a.i.) and commercially formulated clethodim (240 g, a.i./3.8 l) to determine the influence of formulation on stability. The research was initially conducted in the dark to determine the effect of acid catalysis on clethodim degradation. Experiments were also conducted under artificial UV lamps to determine the effect of photocatalysis on clethodim degradation at pH 5, 6, and 7. Clethodim was quantified by high-pressure liquid chromatography (HPLC).[7]

B. INFLUENCE OF ADJUVANTS

Experiments were conducted to determine the influence of five adjuvants on the photodegradation of technical and formulated clethodim. LI700* (a mixture of phosphatidylcholine and methylacetic acid); CC-15943 and XE-1167;** Dash® (petroleum hydrocarbons, napthalene, and oleic acid); and Agrioil®*** (polyoxyethylene esters) of polyol, fatty acids, and polyoxyalkylene ethers. The treatments were conducted under sunlight and artificial UV lamps. Experimental particulars have been published.[7]

C. RELATIVE EFFICACY OF CLETHODIM AND POLAR DEGRADATION PRODUCTS

The purpose of this research was to determine the relative efficacy of technical-grade clethodim and polar degradation products of technical clethodim. An aqueous solution of clethodim, pH 5, was exposed to artificial UV light for 4 h, after which the solution was partitioned with acetonitrile. Parent clethodim was assayed by HPLC and fractionated from polar degradation products by liquid partitioning with hexane:water (5:1, v/v). The aqueous solution was partitioned three times and the hexane phase discarded each time. The quantity of clethodim that degraded was calculated by comparing the resulting concentration of parent clethodim to the initial concentration. Mixtures of clethodim and clethodim degradation products were mixed in five proportions — 1:0, 3:1, 1:1, 1:3, and 0:1 — and applied to grain sorghum seedlings for visual efficacy evaluation. Two series of experiments were conducted. One series was initiated at 20:00 h in a greenhouse to permit approximately 10 h of darkness during which maximum plant uptake could occur without further loss from photodegradation. A second series of treatments was initiated at 8:00 h on a clear day.

D. CLETHODIM ABSORPTION

Experiments were conducted to compare foliar absorption of ^{14}C-clethodim using each of the five previously mentioned adjuvants. Absorption studies were conducted using ring-labeled ^{14}C-clethodim and polar degradation products of ^{14}C-clethodim. Degradation and separation procedures were similar to those previously described. Experimental details have been published.[2]

III. RESULTS AND DISCUSSION

A. MECHANISM AND RATE OF CLETHODIM DEGRADATION

Results indicate that clethodim is acid labile and that total clethodim recovery declined over the 20-h period.[7] Clethodim loss was 37, 8, and 0% at pH 5, 6, and 7, respectively (Figure 1), at 20 h in the dark. Furthermore, clethodim was shown to be labile under artificial UV light, with clethodim degradation after 20 h being 100, 100, and 99% at pH 5, 6, and 7, respectively. Degradation rates and products were similar for technical and formulated clethodim.[7] Particulars regarding acid- and photocatalyzed degradation have been published.[7] Separation and identification of degradation products is continuing.

These results indicate that even though the acid or protonated form of clethodim is the preferred species for foliar uptake and absorption, the presence of this species in acid aqueous environments renders it unstable. Furthermore, upon exposure to UV light, the clethodim in these aqueous solutions (emulsions) degraded rapidly. These properties are undesirable, not only because they would limit efficacy once the solution is applied, but also because the lability of acid aqueous solutions will limit, if not preclude, marketing premixes and/or

* Loveland Industries, Greeley, CO.
** Valent U.S.A. Corp., Walnut Creek, CA.
***ChemNut, Inc., Albany, GA.

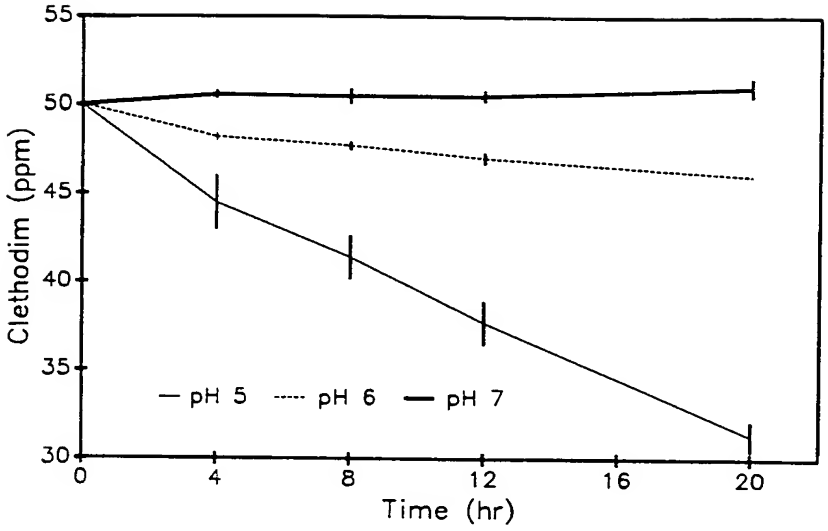

FIGURE 1. Influence of pH on technical clethodim degradation in the dark at 22°C. (From Falb et al., *J. Agric. Food Chem.*, 38, 875, 1990. With permission.)

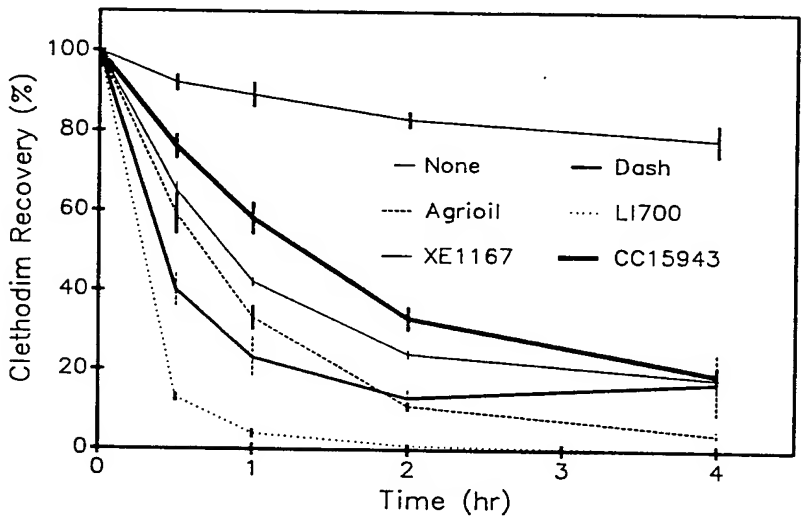

FIGURE 2. Influence of five adjuvants on the degradation rate of 2 EC clethodim in sunlight. (From Falb et al., *J. Agric. Food Chem.*, 38, 875, 1990. With permission.)

ready-to-use formulations of these herbicides. These results may explain why less than half of the herbicide applied actually entered the plant.

B. INFLUENCE OF ADJUVANTS

All five adjuvants increased the rate of clethodim degradation by two- to sevenfold, compared to the no-adjuvant control (Figure 2). Experiments were conducted in buffered (pH 7) solutions. Since clethodim is relatively stable in the dark at pH 7, the predominant

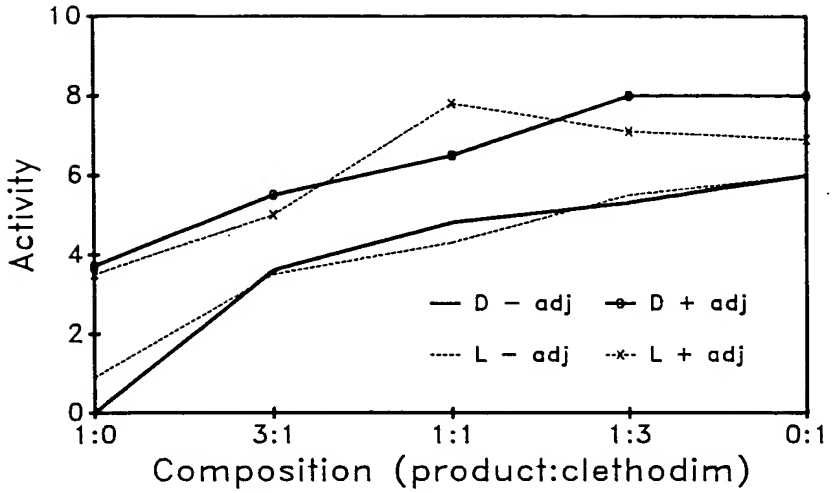

FIGURE 3. Relative activity of clethodim and polar products of clethodim, with and without adjuvant.

degradation mechanism appears to be light dependent. In fact, clethodim degradation in sunlight and under artificial UV light conditions were similar. Half-lives and particulars related to adjuvant effects on clethodim degradation have been published,[6,7] and indicate that degradation rates were in the descending order of: LI700 = Dash > XE1167 = Agrioil > CC15943 > no adjuvant.

Research has clearly demonstrated that the addition of an adjuvant, preferably crop oil concentrate, is required for optimum activity of cyclohexanedione herbicides.[9-11,14] However, our research indicates that the addition of several commonly used adjuvants enhanced photodegradation. The results indicate that macroaggregation of lipophilic clethodim molecules occurs. This could increase the probability of chain reactions, and thus clethodim degradation by free radical mechanisms or energy transfer. Also, since adjuvant addition is required, a prudent approach to adjuvant selection is to select compounds which do not promote degradation processes and which protect the active molecule.

C. RELATIVE EFFICACY OF CLETHODIM AND POLAR DEGRADATION PRODUCTS

Research results indicate that clethodim was more active than the polar product on sorghum seedlings, particularly in the absence of Dash (Figure 3). The activity of treatments containing clethodim was only about 40% greater when Dash (1%, v/v) was added, compared to no additions of Dash. When only polar product(s) of clethodim were applied, little or no activity was observed in the absence of Dash. However, when Dash (1%, v/v) was added, activity was approximately 75% compared to the application of similar concentrations of clethodim without Dash. Initiating experiments in the greenhouse at 8:00 h vs. 20:00 h had little impact on the activity of clethodim or its polar product(s).

The results show that not only does degradation occur, but that at least some of the degradation products are herbicidally active. They also indicate that because of the polar nature of the products, addition of adjuvant is essential for absorption and subsequent herbicide activity. Therefore, adjuvants capable of mediating the uptake of polar products might enhance the activity of cyclohexandione herbicides, especially when these herbicides are applied under conditions favorable for degradation of the parent herbicide.

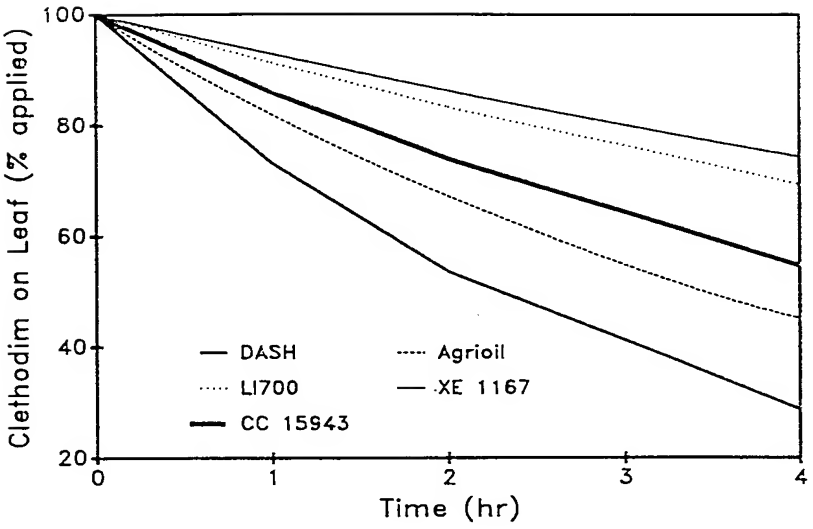

FIGURE 4. Influence of five adjuvants on foliar absorption of ^{14}C-clethodim.

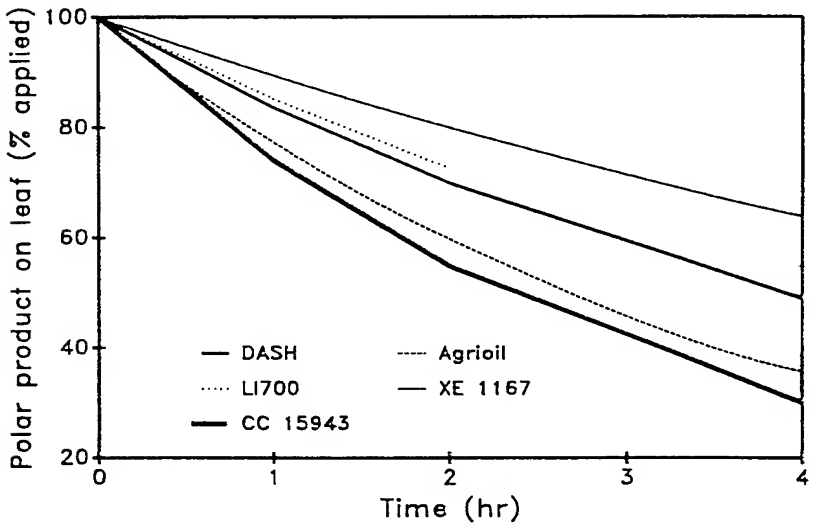

FIGURE 5. Influence of five adjuvants on foliar absorption of polar degradation products of ^{14}C-clethodim.

D. CLETHODIM ABSORPTION

The data indicate that foliar uptake of clethodim was greater (both rate and total amount) with Dash compared to the other adjuvants tested (Figure 4). These data indicate that the uptake of clethodim is more rapid with the addition of Dash than with the other adjuvants, which may contribute to the performance observed with the adjuvant in field tests despite the fact that photodegradation of clethodim in the presence of Dash is more rapid than in the presence of several of the other adjuvants tested, particularly CC 15943.

Foliar uptake of polar degradation products of ^{14}C-clethodim was greater with Agrioil and CC 15943 than with Dash (Figure 5). In field trials on johnsongrass, clethodim efficacy

with CC 15943 and XE 1167 have been comparable to efficacy with DASH. Even though foliar uptake of clethodim does not appear to have been as rapid with CC 15943, foliar uptake of polar degradation products was more rapid and the photodegradation rate with this adjuvant was less than with Dash.

IV. SUMMARY

Results of this research indicate that the lability of clethodim when exposed to acid conditions and/or UV light may significantly affect the efficacy of this herbicide. They also demonstrate that the use of adjuvants with cyclohexanedione herbicides may present effects that are confounded since they appear to be essential for activity, yet often enhance photodegradation. It is apparent that adjuvant selection with these herbicides is a critical decision and may impact the efficacy of herbicide application in ways previously unrecognized. When selecting adjuvants for use with cyclohexanedione herbicides such as clethodim, one should consider compatibility, photodegradation, pH, and absorption characteristics. A prudent approach to adjuvant management with these herbicides would be to select adjuvants which promote herbicide stability and concurrently promote rapid foliar absorption.

ACKNOWLEDGMENTS

This research was supported by State and Hatch funds (H-1407 and H-1403) allocated to the Georgia Agricultural Experiment Stations and by a grant from Valent U.S.A. Corp., Walnut Creek, CA. Analytical services were provided by the Complex Carbohydrate Research Center, University of Georgia, Athens, which is supported in part by Department of Energy Grant DE-FG09-87ER13810 as part of the United States Department of Agriculture/Department of Energy/National Science Foundation Plant Science Centers program.

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Chapter 19

COMPARISON OF THE CYCLIC ETHER-ALCOHOL TETRAHYDROFURFURYL ALCOHOL TO OTHER KNOWN SOLVENTS

K. J. Doyel, W. J. McKillip, C. C. Shin, and D. A. Rickard

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ABSTRACT

Tetrahydrofurfuryl alcohol (THFA 2®-tetrahydrofuryl methanol), as an agrichemical adjuvant that has seen limited commercial applications. THFA's underexploited status is believed due to a lack of publicly available data regarding its characteristics.

Inherently low toxicity, low volatility, biodegradability, and high solvency in both organic and aqueous systems make THFA an attractive candidate for use with agrichemicals. Studies were performed to characterize the chemical with respect to the utility of THFA in agrichemical adjuvant applications.

The results are discussed in comparison to other widely used solvents and carriers. The data presented should provide formulators with another option when selecting formulation chemistry for current or experimental active ingredients.

I. INTRODUCTION

If one endeavors to create a perfect solvent for use as a coupling agent with various active materials, it would have the characteristics shown in Table 1.[2]

The problem, however, is that few commercial compounds contain all of these attributes. Most commercial adjuvants have been chosen on the basis of cost and availability. Many of these products are well-known solvents and oils. Today, formulators and producers are finding utility in lesser known solvents based on the different cyclic compounds of pyrrole and furan.[3,5]

THFA is a colorless organic solution having the structure shown in Figure 1. THFA is unique in that its structure contains elements of an ether, an alcohol, and a cyclic molecule. THFA is produced commercially by the catalytic hydrogenation of furfural, the furan aldehyde. Furfural is obtained industrially from pentosan containing agricultural byproducts such as corncobs, rice hulls, oat hulls, cottonseed hulls, and sugarcane bagasse.[5]

THFA is unique in that it is easily miscible in water and most organic solvents. In addition, the product has low volatility and can aid in retarding evaporation. Table 2 contains a list of the physical properties of THFA.[5]

Recent findings of new toxicity levels of various solvents have caused some concern with manufacturers and formulators. To combat this, manufacturers are reformulating with different solvents which have lower toxicity characteristics. THFA is one such chemical that is receiving more attention based on its positive toxicity characteristics. Figure 2 compares THFA oral toxicity in rats to oral toxicity levels of other well-known EPA-exempt (40 CFR 180.1001) solvents. The table provides a direct comparison of one parameter, which may be different in other species or conditions.[6,7]

The purpose of this paper is twofold: (1) to introduce the reader to a solvent which has had little or no exposure in the area of agrichemicals and (2) to provide some basic experimental data on the solubility of THFA with well-known agricultural active ingredients.

II. MATERIALS AND METHODS

The experiment consisted of determining (1) the maximum solubility range of THFA with the chosen active ingredient, (2) the maximum solubility of the THFA/active ingredient mixture in water, and (3) if a 1:100 ratio of mixture to water would result in a stable solution.

Selection of the active ingredients for this experiment was based on the criteria of commercial use, lack of aqueous solubility, and general class of material. Table 3 lists the 52 active materials tested in this experiment. The materials were obtained from Chem Service, Inc., West Chester, PA, and the THFA, from QO Chemicals. All material was of commercial

TABLE 1
Characteristics of Solvents

Low toxicity
Low phytotoxicity
Low volatility
High flash point
Ability to solubilize many different active materials
Coupling ability
Biodegradability
Cost effectiveness

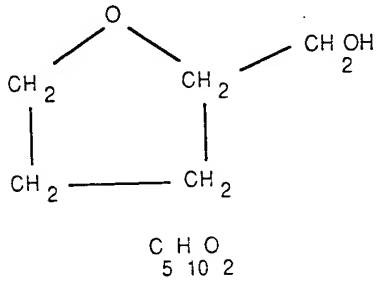

FIGURE 1. Chemical structure of tetrahydrofurfuryl alcohol (THFA).

TABLE 2
Characteristics of Tetrahydrofurfuryl Alcohol

Boiling point	178°C, 352°F
Freezing point	−80°C, −112°F
Vapor pressure	0.4 mmHg @ 20°C
Density	8.76 lbs/gal
Flash point	165°F TCC method
Soluble in	Water, alcohols, aromatics, esters, ethers, ketones, and chlorinated hydrocarbons
Insoluble in	Coconut, cottonseed, and peanut oils, anthraquinone, dextrose, and paraffinic hydrocarbons

EPA exemption from tolerance as a solvent/cosolvent with no limits per 40CFR 180.1001
Not phytotoxic in concentrations up to 25%

purity and was obtained uninhibited in order to eliminate the introduction of other variables to the test. The water for dilution was ordinary tap water provided by the city of Memphis, TN.[1,4]

A. THFA/ACTIVE MATERIAL SOLUBILITY

One gram of the selected active material was weighed and placed into a tared 20-ml vial. One gram of THFA was added to produce a 1:1 solution, and the vial was agitated in order to mix the two materials. The solution was visually inspected to determine solubility. If initially the THFA appeared to solubilize the material, the mixture was allowed to stand for 10 min and then reinspected to assure solubility. If the material was not completely solubilized, an additional 2g of THFA was added to produce a 1:1 solution. The same steps were repeated to visually inspect for solubility. The protocol was repeated at concentrations

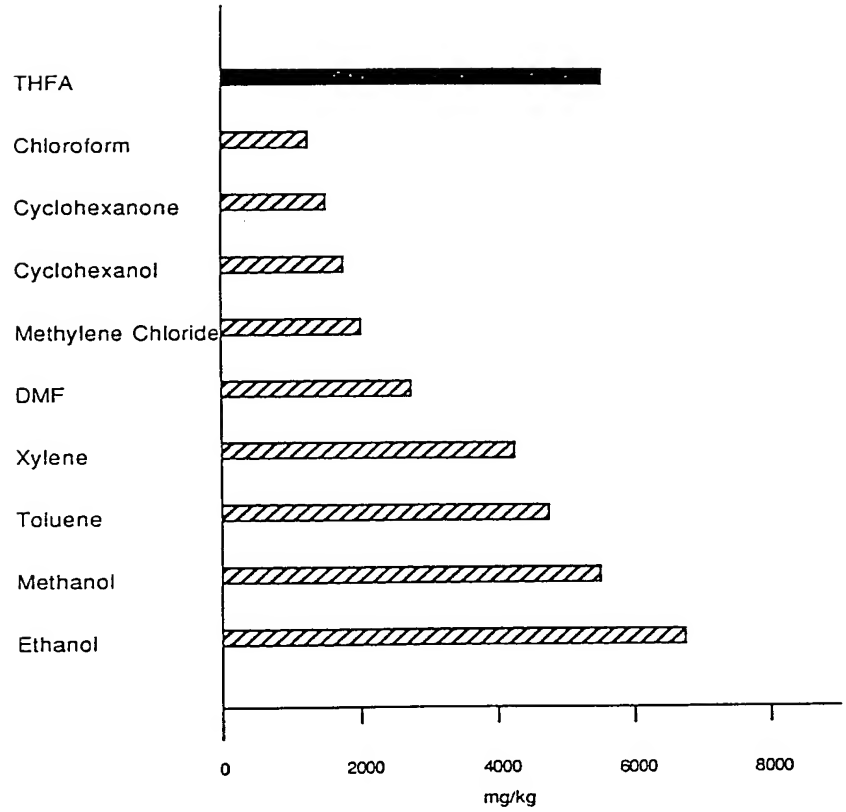

FIGURE 2. Toxicity levels of various solvents (oral toxicity to rats LD_{50}).

of 1:9, 1:19, 1:99, and 1:>99 until the material solubilized in the THFA. The temperature was not controlled in the experiment, and was assumed to be 25°C.

B. WATER SOLUBILITY IN THFA/ACTIVE MATERIAL MIXTURE

This part of the experiment was designed to measure the coupling ability of the THFA. The solution of maximum solubility in part A was used for this experiment. For mixtures greater than a 1:10 ratio, enough THFA was added to bring the solution to a 10% mixture (11 g total), so as to have enough material for this experiment and part C. For mixtures of 1:19, 1:99, and 1:>99 ratios, 10 g of each solution, as is, were used for this experiment.

The 10 g were placed in a stirred vial. Water was added in increments of 0.25 ml to the mixture. The solution was visually inspected to determine the quantity of water necessary to initiate precipitation of the active ingredient and the volume of water was recorded for each mixture.

C. THFA/ACTIVE MIXTURE SOLUBILITY IN 1:100 RATIO WITH WATER

This part of the experiment was designed to determine the coupling ability of THFA in a common commercial dilution ratio (1:100) with water. The mixtures used in part B were also used in this experiment. One gram of the mixture was slowly added with a micropipette to 100 g (or 100 ml) of water. At the first sign of precipitation, the solution was stirred and

TABLE 3
Materials Used In Test

Class	Material name
Acetalmide/anilide	Butachlor
	Propachlor
	Propanil
Arsenicals	MSMA
Benzothiazole	Bentazon (Basagran)
Carbamate	Carboxin
	Carbofuran (Furadan)
Carbamates/thiocarb	Butylate
	EPTC
	Molinate
	Triallate
Chloracentanilide	Metolachlor
Chlorinated HC	Endosulfan
Dinitroaniline	Pendimethalin
Diphenyl ether	Diclofop-methyl (Hoelon)
Halogenated phenols	PCP (Pentachlorophenol)
Halogenated acid/derivatives	Chloramben (Amiben)
	DCPA (Dacthal)
Miscellaneous	Triadimefon (Bayleton)
	Iprodione (Rovral)
	Etridiazole (Terrazole)
Nitrogen compounds	Bromoxynil
	Maleic hydrazide
Phenoxy acids/derivatives	2,4-DB
	MCPA
Phosphate	Isofenphos
	Isazophos (Miral)
	Naled
	Phenamiphos
Pyrethroid	Allethrin
Pyridazinone	Norflurazon (Zorial)
Quaternary ammonia	Diquat
Repellents	Deet
	MGK-R-11
Thiocarbamates	Benomyl
	Maneb
	Zineb
Thiophosphates	Diazinon
	Dimethoate
	Disulfoton
	Fonofos (Dyfonate)
	Azinphos-methyl (Guthion)
	Malathion
Triazines	Atrazine
	Cyanazine
	Prometon
	Simazine
Triazole	Propiconazole (Tilt)
Urea derivatives	Bromacil
	Diuron
	Fluometuron
	Terbacil

TABLE 4
Solubility (High) of Various Materials in Tetrahydrofurfuryl Alcohol (25°C)

1:1 Ratio		1:3 Ratio	
Allethrin	Isazophos	Azinphos-methyl	MCPA
Butachlor	Isofenphos	Bentazon	PCP
Butylate	MGK-R-11	Bromoxynil	Pendimethalin
Deet	Malathion	Chloramben	Propachlor
Diazinon	Metolachlor	Diclofop-methyl	Propanil
Dimethoate	Molinate	EPTC	Propiconazole
Disulfoton	Naled	Endosulfan	Triadimefon
Etridiazole	Phenamiphos		
Fonofos	Triallate		

Note: Ratio, active to THFA.

TABLE 5
Solubility (Low) of Various Materials in Tetrahydrofurfuryl Alcohol (25°C)

1:9 Ratio	1:19 Ratio	1:99 Ratio	1:>99 Ratio
2,4-DB	Diuron	Atrazine	Benomyl
Bromacil	Iprodione	Carbofuran	DCPA
Carboxin		Fluometuron	Diquat
Cyanazine		Norflurazon	MSMA
Maneb		Prometon	Maleic hydrazide
Terbacil			Simazine
			Zineb

Note: Ratio, active to THFA.

reevaluated. This procedure was repeated until the mixture either went into solution or formed on obvious precipitant.

III. RESULTS AND DISCUSSION

A. THFA/ACTIVE MATERIAL SOLUBILITY

The results of the solubility of various active materials with THFA are shown in Tables 4 and 5. The results show the excellent solubility performance of THFA, with over 60% of the materials tested exhibiting high solubility. In general, phosphates, thiophosphates, carbamates, and acetamides exhibited high solubility with THFA. Thiocarbamates, triazines, and urea derivatives evidenced a lower affinity to dissolve in THFA.

B. WATER SOLUBILITY IN THFA/ACTIVE MATERIAL MIXTURE

For our experiment, we chose active materials which have a low solubility in water.[4] From part A, we know that the binary system of THFA and active material will show a high degree of solubility. From a practical standpoint, this can be and has been, used as an acceptable spray mixture for ULV applications. However, a much better mixture may be formed by diluting the binary system with water. Parts B and C of this experiment are attempts to understand and quantify this ternary system.

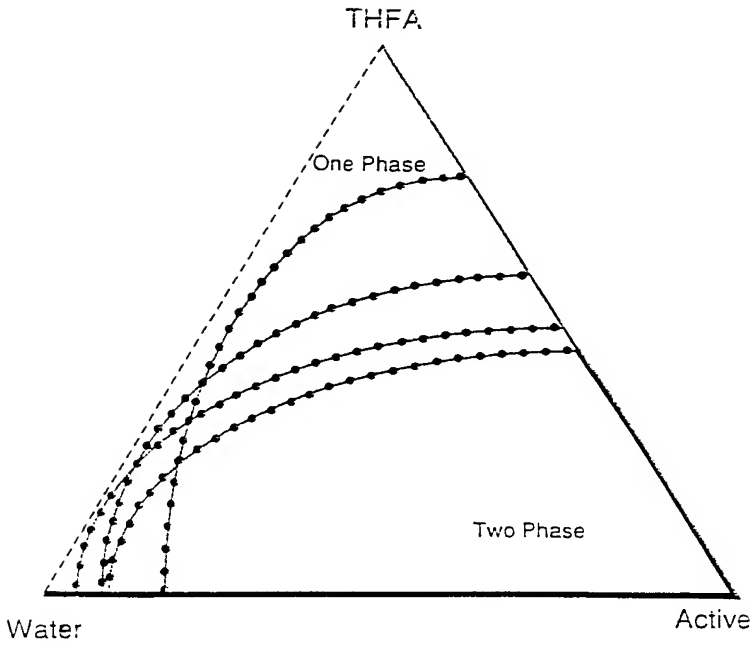

FIGURE 3. Part A — solubility curves of three components.

In order to understand what our experiment is revealing, we must first review what our system looks like by constructing a triangular phase diagram (Figure 3). In this figure, we know that THFA and water are soluble in all portions (denoted by dashes), and that water and active materials are insoluble in portions containing less than 99% water (denoted by thick solid lines). From part A, we determined that blends of THFA and active materials are soluble in varying proportions, depending on the active material chosen (denoted by the thin solid line). The key unknown in this experiment is that we have no information on what shape the solubility curve will take in the interior of the triangle (denoted by the dotted lines).

In Part B of the experiment, we are adding water to the mixture. Figure 4 shows how the addition of water changes the concentration so that the concentration at any point in time will exist somewhat along the tie line drawn in the figure. Saturation of the mixture and precipitation will occur if the tie line intersects the solubility curve. In this experiment, we are testing only one binary concentration of THFA/active material. One could reproduce a portion of this curve by selecting a number of initial starting concentrations and plotting the saturation points on the diagram after the addition of water. Part C of the experiment concerns the area outlined in Figure 5. This area was tested to determine if precipitation would occur in a commonly used dilution ratio (1:100). In addition, it can provide some information about the back side of the solubility curve. A given mixture of the three substances may have a very unique curve that allows two areas of complete solubility (as shown in Figure 4, lower tie line), one at low water concentrations and one at higher water concentrations.

The results of a portion of part B of the experiment are shown in Table 6. These results show that the addition of THFA will couple the active material up to a certain point. THFA

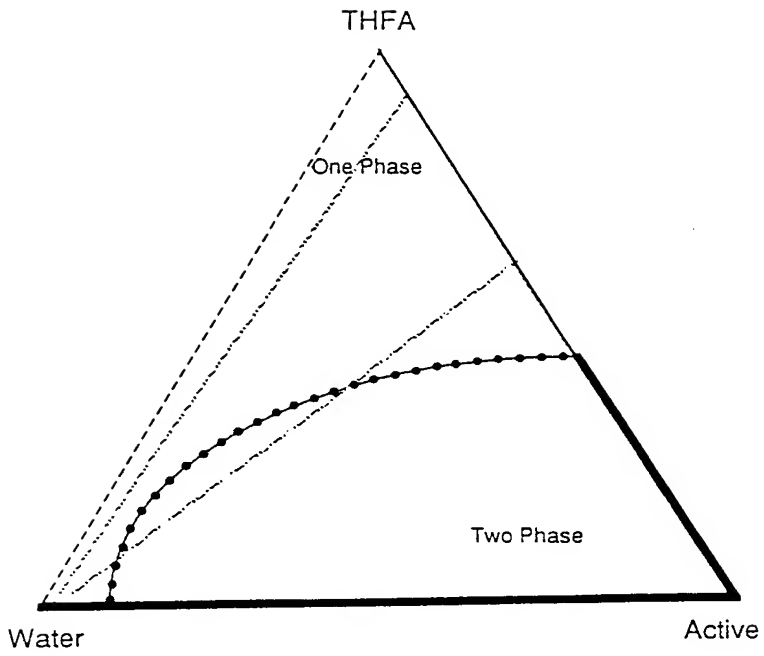

FIGURE 4. Part B — tie lines–water addition to THFA/active mixture.

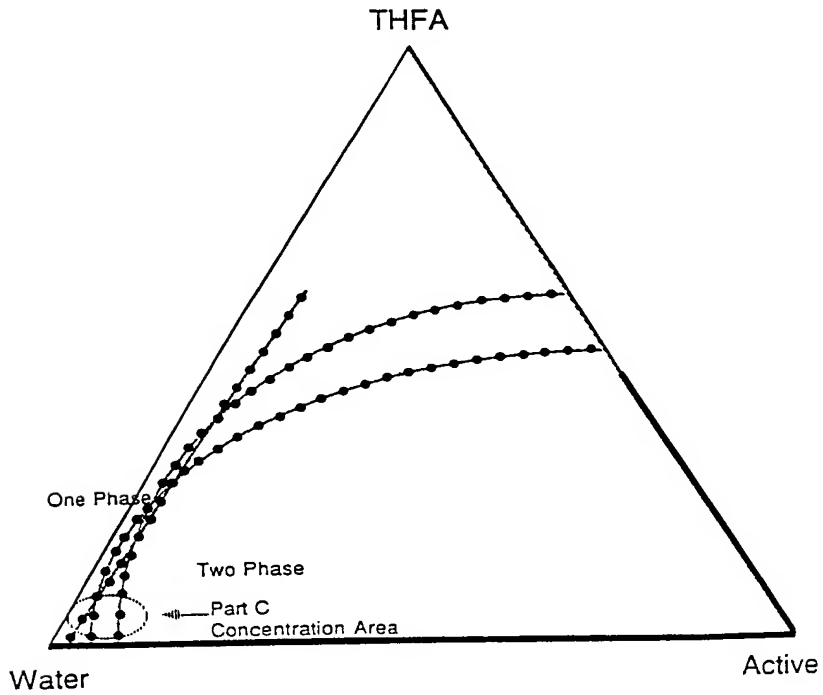

FIGURE 5. Part C — solubility of mixture in 1:100 dilution.

TABLE 6
Solubility of Water in THFA/Active Mixture

Chemical name	Active solution (%)	Water solubility (water:mixture)
2,4-DB	10	3:10
Atrazine	1	Soluble
Azinphos-methyl	10	7:10
Bentazon	10	Soluble
Bromacil	10	3.25:10
Bromoxynil	10	4.25:10
Butachlor	10	8.25:10
Butylate	10	3:10
Carbofuran	1	6:10
Carboxin	10	8:10
Chloramben	10	Soluble
Cyanazine	10	3:10
DCPA	<1	3.75:10
Deet	10	10:10
Diazinon	10	2.25:10
Diclofop-methyl	10	4:10
Disulfoton	10	4:10
Diuron	5	5:10
EPTC	10	5:10
Endosulfan	10	5:10
Etridiazole	10	5:10
Fluometuron	1	6:10
Fonofos	10	5:10
Iprodione	5	3.75:10
Isazophos	10	6.75:10
Isofenphos	10	5.5:10
MCPA	10	10:10
MGK-R-11	10	3:10
Malathion	10	6.5:10
Metolachlor	10	2.75:10
Molinate	10	6:10
Naled	10	10:10
Norflurazon	1	5.5:10
PCP	10	9:10
Pendimethalin	10	2.5:10
Phenamiphos	10	7.25:10
Prometon	1	5:10
Propachlor	10	4.75:10
Propanil	10	10:10
Propiconazole	10	5:10
Terbacil	10	4:10
Triadimefon	10	9:10
Triallate	10	5:10

appears to do well in having some coupling effect on the broad range of substances tested. An interesting experiment beyond the scope of this study would be to continue the addition of water to determine if, and at what concentration, the material would resolubilize, and whether this new concentration would be optimal for commercial applications.

C. THFA/ACTIVE MIXTURE SOLUBILITY IN 1:100 RATIO IN WATER

As discussed, a point was picked to determine whether THFA would couple the active ingredient in a large dilution of water. Table 7 shows a compilation of the materials that

TABLE 7
THFA/Active Mixture Solubility in 1:100 Ratio with Water

Chemical	Mix concentration active:THFA	Water solubility of active
Soluble		
Allethrin	1:10	Insoluble
Atrazine	1:99	33 ppm
Bromacil	1:10	815 ppm
Carbofuran	1:99	700 ppm
Deet	1:10	Insoluble
Diuron	1:19	42 ppm
Fluometuron	1:99	90 ppm
Naled	1:10	Insoluble
Prometon	1:99	620 ppm
Partially soluble		
Benomyl	1:>99	Insoluble
Bentazon	1:10	Insoluble
Chloramben	1:10	700 ppm

were solubilized. THFA appears to be somewhat effective in getting some of the materials to solubilize in water. As previously discussed, it would be interesting to generate solubility curves for the materials in order to determine an optimal concentration for commercial use.

IV. CONCLUSIONS

The results of this study show that:

1. THFA has some unique characteristics which may be of interest to the manufacturer or formulator.
2. THFA alone has good solubility performance with many common active materials.
3. THFA can couple these materials into water; however, three-component equilibrium solubility diagrams should be constructed to determine optimal concentrations for commercial use.

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Section II

Regulation and Importance; Environmental Effects; Spray Deposition and Dissipation; Soil Adjuvants; Organosilicone Surfactants, Oils, and Emulsifiers

Chapter 20

REGULATION OF PESTICIDES AND INERT INGREDIENTS IN PESTICIDE PRODUCTS

Edwin F. Tinsworth

The Environmental Protection Agency (EPA) regulates pesticides under two statutes: the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA). Recent amendments to FIFRA, proposed amendments to FFDCA and continuing debate in Congress regarding food safety make discussions of registration, reregistration, and special review as they relate to both active and inert ingredients in agrichemicals timely. The following is a summary of the EPA's authority to regulate pesticides and adjuvants; the three programs — registration, reregistration, and special review — that are the backbone of pesticide programs at the EPA; and what the future may hold in terms of these processes.

FIFRA requires that all pesticide products sold or distributed in commerce be registered by the EPA. It further requires that the EPA determine that the product, when used according to directions, will not pose unreasonable risk to human health or the environment, taking into account the risks and benefits of the chemical's use. In addition, FIFRA requires the EPA to reregister existing pesticides.

Under the FFDCA, the EPA is authorized to establish tolerances of pesticide residues on raw agricultural commodities (RACs) and food additive regulations for pesticide residues in processed foods.

Prior to the establishment of the EPA, the Food and Drug Administration (FDA) was responsible for establishing tolerances and food additive regulations. In 1961, the FDA published a notice that each component of a registered pesticide product, including inert ingredients, was a pesticide chemical and subject to the requirements of tolerances under the FFDCA. In 1969, they established a policy requiring that certain minimal toxicity data be provided on inert ingredients and stating that the need for data depicting the residues of the inert ingredients in or on RACs and processed food would be dependent on the substance's toxicity. The FDA policy also provided for a less formal clearance process of inerts that were deemed to be generally recognized as safe (GRAS).

The definition established by the FDA clearly indicates that the EPA may set tolerances and food additive regulations for inert ingredients in pesticides. Further, regulations under FIFRA which set forth the data generally required to assess a pesticide's risk also state that the EPA can require data on inert ingredients in pesticide products. Additionally, the EPA's authority to require data extends to end use-formulated products plus any recommended vehicles and adjuvants not part of the product, but mixed with the product at the time of its use.

Despite the fact that the EPA can regulate adjuvants under existing and past authority, little testing has been required to date. About 50% of the approximately 1200 existing inert ingredients used in pesticide products have been cleared for use under the FFDCA. Over 100 of these have been deemed GRAS by the FDA in the past. Until recently, most regulatory actions and data requirements under FIFRA for food-use and non-food-use pesticides have focused on the potential risks posed by the pesticide active ingredients in formulated products. The basic exception is that the EPA requires product chemistry and acute toxicity testing on the formulated, or end-use product, thereby characterizing the acute risks of the combination of active and inert ingredients that make up that product.